PROGRESS REPORT OF THE WOYLIE CONSERVATION RESEARCH PROJECT

Diagnosis of recent woylie (*Bettongia penicillata ogilbyi*) declines in southwestern Australia

A report to the Department of Environment and Conservation Corporate Executive

Photo courtesy of Sabrina Trocini

January 2008

Principal Project Collaborators:

Murdoch University

Perth Zoo

australian wildlife conservancy

Government of Western Australia Department of Environment and Conservation

Principal Project Funding Sources

'Saving our Species' Biodiversity Conservation Initiative

Department of Environment and Conservation

South West Catchments Council

Executive Summary

Recent woylie declines – situation summary

The woylie (*Bettongia penicillata ogilbyi*) had a distribution across much of Australia prior to settlement by Europeans. By the 1960's the woylie was reduced to three isolated remnant populations in southwestern Australia (Upper Warren [principally Perup], Tutanning and Dryandra) (Figure 1). Fox control and woylie reintroductions began in the 1970's. Since 1996, these activities have been expanded and strategically managed as part of the '*Western Shield*' conservation program (Orell, 2004). Cumulatively, these efforts resulted in a dramatic recovery that culminated in the woylie being the first Australian mammal to have its conservation status downgraded as a result of it being delisted from Commonwealth and State conservation lists (Endangered / Threatened) in 1996 (Start *et al.,* 1998).

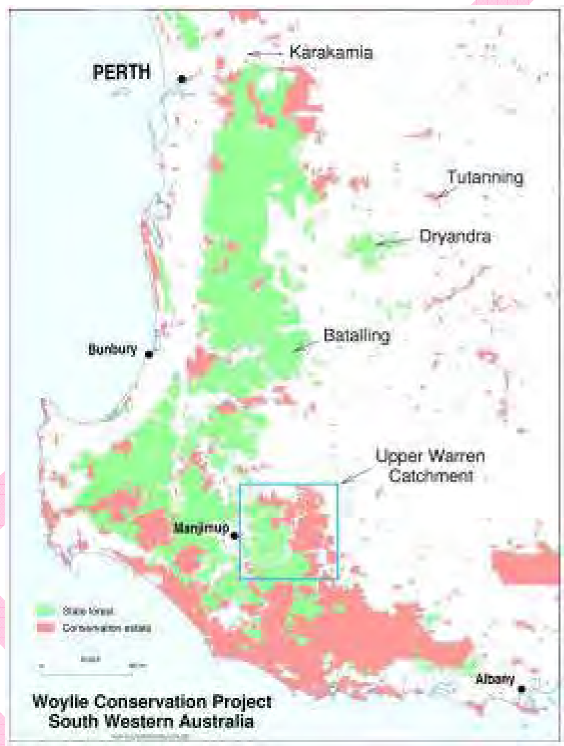


Figure 1. The location of important woylie populations.

Since 2001, however, woylie populations throughout southwestern Australia have undergone rapid and substantial declines. For example, capture rates from long-term monitoring and research has indicated that the woylie population at Dryandra declined by 93% between 2000 and 2006. The Upper Warren population(s) have so far undergone a median decline of 95% between 2002 and 2007 (Figure 2). The woylie population at Batalling (a 1982 reintroduction site east of Collie) has declined by 97% between 2002 and 2007. Evidence from multiple sources including live trapping, sandpad surveys, woylie nest and digging densities and spotlight surveys all concur and demonstrate that these declines are real (Wayne, 2006). A concurrent woylie decline has also been observed in South Australia (Venus Bay Peninsula); while South Australian island populations appear to have remained relatively stable (Jason VanWeenan, pers. comm.).

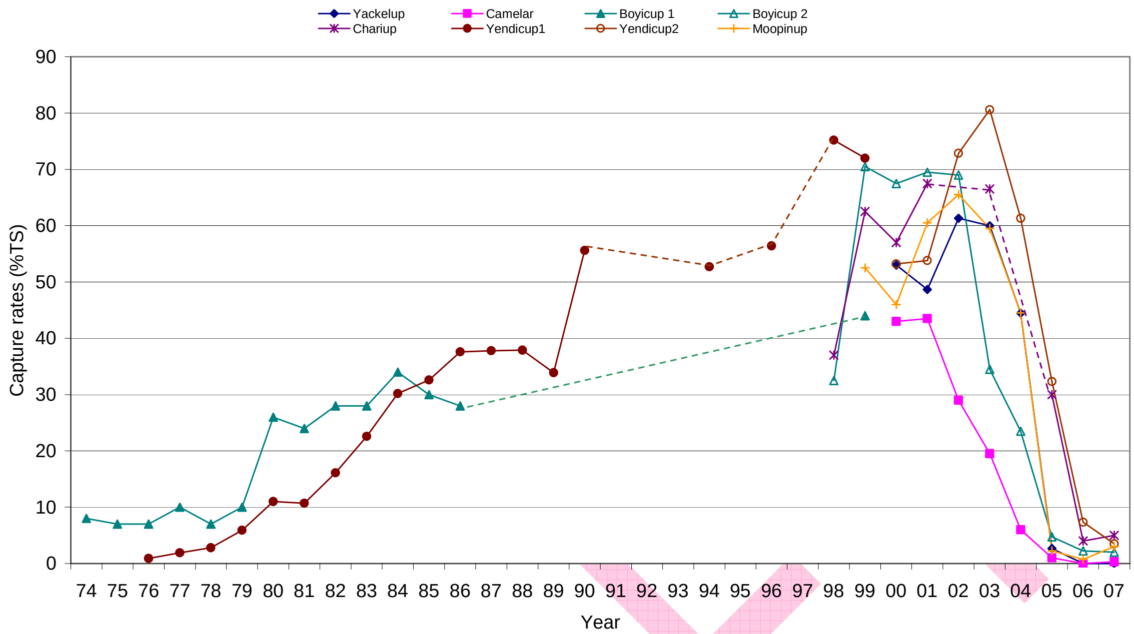


Figure 2. Capture rates of woylies over time on each of the Upper Warren Fauna Monitoring transects in southern and central areas of Perup Nature Reserve.

Note: Transect names with the suffix 1 and 2 distinguish relatively similar transects within the same area with slightly different methodologies surveyed by different groups (e.g. slightly different transect locations, trapping frequency, etc).

The dashed lines are indicative trends during the intervening periods between trapping events in non-successive years

In general, the largest and most substantial woylie populations have undergone substantial declines while the very small and/or low density, often isolated populations appear to be less affected. Overall, an estimated 70-80% decline in woylie numbers has occurred in Australia over five years. These declines are still continuing and there is little evidence of signs of a recovery (Freegard, 2007). Introduced predators (fox and cat) and habitat loss were among the principal factors thought responsible for historical declines (Burbidge and McKenzie, 1989; Start *et al.,* 1995). There also is some anecdotal evidence that disease may have caused the decline of many mammal species (including the woylie) in Western Australia in the late 1800s to early 1900s (Abbott, 2006). The purpose of the Woylie Conservation Research Project (WCRP) is to identify the cause(s) of these current declines.

Project background and design

There was a report of a suspected woylie decline at a trapping site in the Upper Warren on 26 October 2005. An initial situation assessment of woylie populations throughout the southwest was followed by an early-response workshop (February 2006), the establishment of the Woylie Conservation Steering Group and the development and commencement of the Woylie Conservation Research Project (WCRP) (July 2006) (Figure 3). Using a decline diagnosis framework broadly based on the 'declining-population paradigm' (Caughley, 1994), the WCRP focussed primarily at the declines in the Upper Warren and consists of three major components;

1. Upper Warren Fauna Monitoring that built on, enhanced and co-ordinated previously independent existing activities,
2. Meta-analysis of existing datasets that were aggregated into a single database, and

3. A Population Comparison Study (PCS) designed to discriminate factors and attributes associated with contemporary declines. The PCS has five main lines of enquiry;
   Woylie components -
      a) woylie density and demographics,
      b) woylie survival and mortality,
   Key putative agents of decline -
      c) predators,
      d) resources, and
      e) disease.

Most of the fieldwork associated with the initial phase of the WCRP was conducted July 2006 to August 2007. Analysing and reporting on the findings will be the focus through to June 2008. This current progress report will help to inform how best to respond to the recent woylie declines beyond the existing commitments (i.e. assist in the development of phase two).

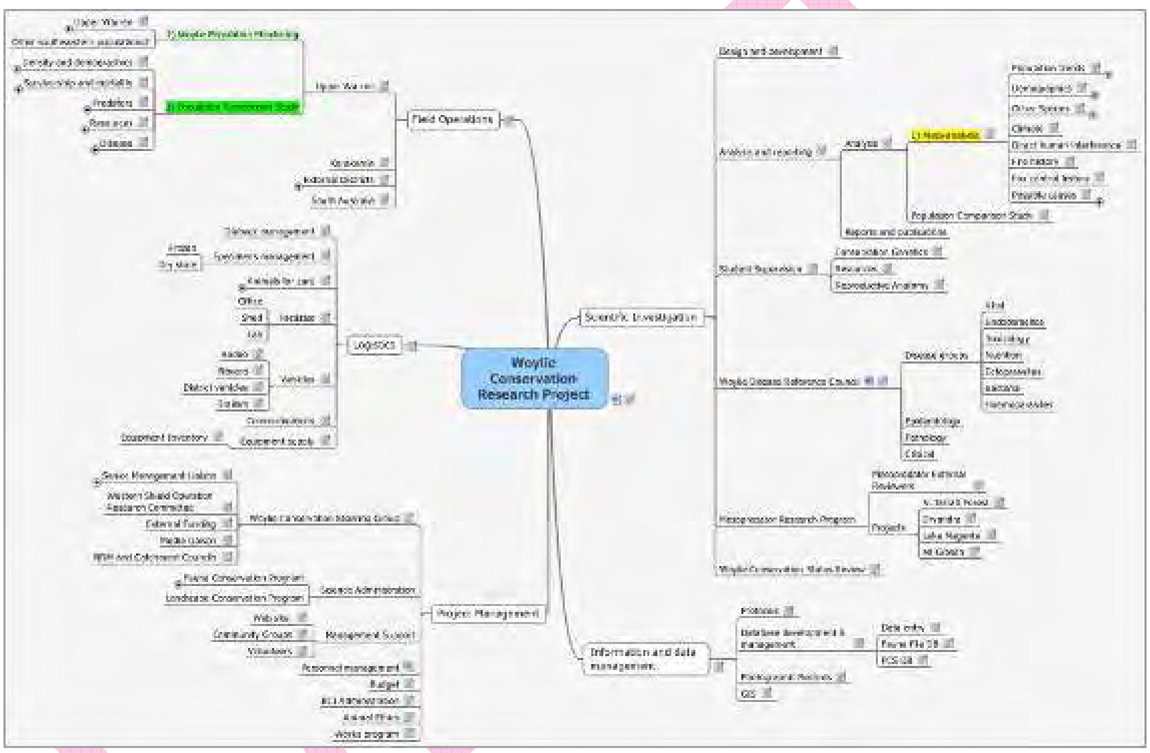


Figure 3. Summary of the Woylie Conservation Research Project management structure and components.

Preliminary inferences (i.e. early hints) of possible causes of recent woylie declines

Although it remains premature to unequivocally identify the cause(s) of the woylie declines, the preliminary results can provide some early hints regarding what the ongoing work is likely to suggest as the likely agents of decline. Factors that are not probably the primary agents of decline include habitat loss / modification, fire, direct human interference from trapping and resources including food. Climate (and extreme weather events) may be associated with woylie declines at Venus Bay Peninsula (VBP), South Australia and cannot be ruled out as a factor in Western Australia. Given the lack of fox activity or density monitoring data associated with most of the observed woylie declines it is not possible to determine whether foxes may be a major agent of decline. This is, however, considered unlikely for the Upper Warren region given that during the WCRP, foxes only accounted for 15% of the implicated primary predators/scavengers associated with observed mortalities and none of the mortalities at the Balban PCS site (which underwent a >80% decline in 12 months) were attributed to foxes.

Increased adult mortality is part of the mechanism driving the rapid and substantial woylie declines. Whether reduced recruitment into the adult (breeding) population is involved in the declines can not yet be established, however, the preliminary evidence suggests breeding rates (prevalence of pouch-young) are not associated with the declines. Emigration of animals elsewhere is not supported by the evidence.

The leading speculative hypothesis (untested) for the cause(s) of the declines is presented (Figure 4). In summary, multiple interactive factors are expected responsible, with disease considered the most likely primary and ultimate agent of decline. The symptoms and unequivocal confirmation of the disease remain elusive at this stage, although some key suspects have been identified including *Toxoplasma*, *Trypanosoma sp. nov.*, possible synergistic effects between the two parasites and the involvement of stressors that may trigger the disease. Other infective agents may also be involved. As a consequence of these disease(s), opportunistic and exploitative predation/scavenging, predominantly by cats, is likely to be the most proximately-related factor associated with the deaths of the woylies. Whether the predation/scavenging occurs on moribund or dead animals that would die regardless, or whether in the absence of predation in general, the woylies would otherwise recover and survive remains unknown.

Shortlist of noteworthy and interesting findings and developments from the WCRP

The Population Comparison Study (PCS) has identified key distinctive differences between woylie populations at Karakamia (which has not declined), and Upper Warren (where substantial declines have occurred) that may relate to the cause(s) of declines. These include;

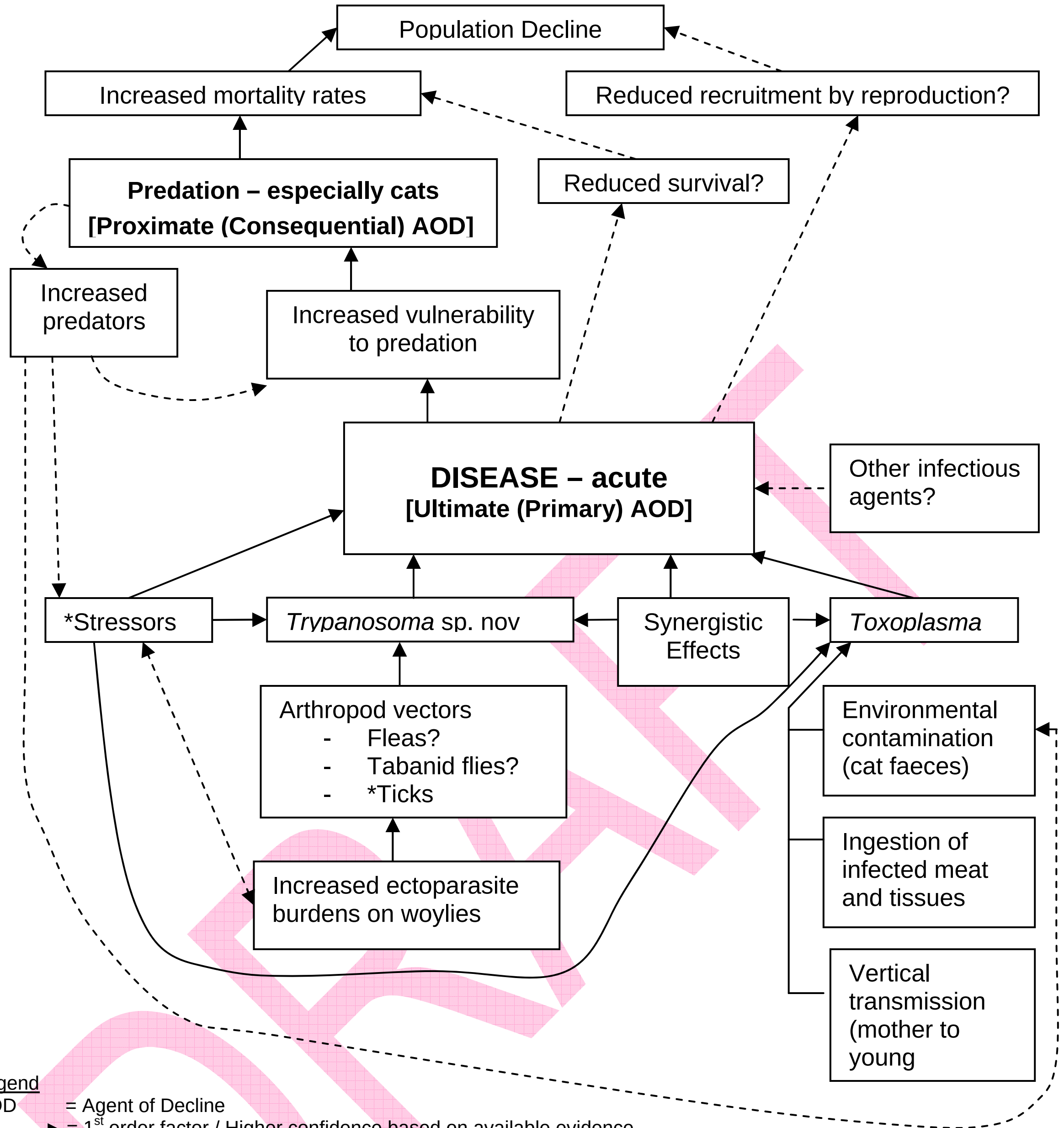
- *Toxoplasma* has not been detected in woylies at Karakamia, but has been detected at Upper Warren.
- *Trypanosoma* is common in the Upper Warren woylie populations (43% prevalence). It has recently been detected at Karakamia, albeit at very low infection levels and prevalence rates relative to the Upper Warren woylie populations.
- Karakamia is effectively free from introduced predators. Cats and foxes are present at Upper Warren.
- Biometric and morphometric data indicates that the high density woylie population at Karakamia, although stable, is very likely resource-limited, in contrast to comparative data from Upper Warren populations, which do not appear resource limited.

Several new species and discoveries have been made as part of the WCRP. These include;

- New species of ectoparasite, endoparasites, haemaparasites, and hypogeal fungi
- New host-parasite associations previously not described
- Evidence of vertical transmission in woylies of *Toxoplasma* from mother to pouch-young.

The collaborations involved in this project are the greatest achievement of the WCRP. These are critical to the success of the project and subsequent conservation outcomes for the woylie. The collaborative capital established as part of this project also has consequences for wildlife research and conservation more broadly. Collaboration developments include;

- Establishment of the Woylie Conservation Steering Group (WCSG) to oversee, co-ordinate and support research and management endeavours to identify the causes of the recent woylie declines and improve the conservation outcomes for the species.
- Project integration with the DEC *Western Shield* Mesopredator Research Program.
- Establishment of the Woylie Disease Reference Council (WDRC) comprised of expertise from Murdoch University and Perth Zoo.
- The principal role of the WCRP and WDRC in the successful support and funding for an ARC linkage project involving DEC and Murdoch University investigating the diseases within threatened mammal fauna throughout Western Australia.



Legend
AOD = Agent of Decline
——▶ = 1st order factor / Higher confidence based on available evidence
- - - -▶ = 2nd order factor / Likely relationships but less evidence available

Not primary factors of decline:
Habitat loss/modification
Fire
Direct human interference (live trapping)
Food resources

Population change – mechanical algorithm for Upper Warren Woylie Population:
0 -ve? 0 +++
Recruitment (immigration + reproduction) – Loss (Emigration + Mortality) ⇒ Population decline

*Possible stressors include:
Predation
Competition
Climate factors / Extreme weather
Concurrent infections
Ectoparasites
Nutrition
Disease reservoirs in sympatric species
High density woylie populations

Figure 4. The leading (untested) hypothesis of the causes of woylie declines in the Upper Warren region based on preliminary and untested inferences.

- The direct involvement of seven university student projects, including three full-time Murdoch University PhD projects with a principal focus on woylies (Resources by Kerry Rodda, Conservation genetics and epidemiology by Carlo Pacioni, Ectoparasites by Halina Burmej) and an Honours project (Reproductive anatomy by Ewa Madon, UWA). Three other Murdoch PhD projects in which the woylie declines constitute a significant component include *Toxoplasma* by Nevi Parameswaran, Endoparasites by Unaiza Parkar and Ectoparasites and bacteriology by Yazid Abdad.

Critical and important collaborative relationships with Australian Wildlife Conservancy and South Australian Government Department of Environment and Heritage.

- Close collaborations within DEC; within the Science Division and between the Science Division and Donnelly District, Warren Region, Nature Conservation Division, Species and Communities Unit, Wellington District, Wheatbelt Region, etc

Operational achievements include;

- A central relational database for fauna in the Upper Warren, including 25,479 woylie records since 1974 to December 2007 created by the aggregation of multiple, previously isolated datasets.
- Field operations handbook providing details on the methodology and protocols for the WCRP, which are also relevant and/or used as part of other DEC activities (monitoring and research).
- Standardisation and synchronization of separate, independent fauna monitoring activities in the Upper Warren region.
- Wildlife forensics course and workshop for DEC and WCRP collaborators.

Interim research and management recommendations

While the Woylie Conservation Research Project (WCRP) is ongoing, some interim and preliminary recommendations for the development of the next phase of research, fauna monitoring, conservation management and project management can be outlined.

The principal research framework includes four themes of development;

1. Phase 1 completion and synthesis
2. Key putative agents of decline
   a) Disease - *Toxoplasma*, *Trypanosoma*, supporting (diagnostic) evidence (field, clinical, pathology, epidemiology), other infectious agents (including priority risks), and dependent follow-up (e.g. synergistic effects)
   b) Predator control experiment (Active Adaptive Management)
   c) Resources
   d) Stressors
3. Species recovery
   a) Conservation genetics
   b) Small population paradigm – limiting factors and risks
   c) Population viability analysis
4. Research management (statistical analyses support, reviews, symposium and workshop, data and information management)

Fauna monitoring recommendations include;

- Suggested programs for Upper Warren and elsewhere
- Improved monitoring protocols (surveillance versus ecological monitoring; target species, predators, other covariates; and trigger points, reporting and response protocols)

Interim and preliminary conservation management recommendations include;

- Conservation status reviews
- Recovery planning
- Improved efficiency to the current fox-control program

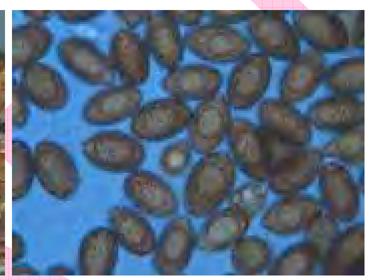
- Data and information management improvements

Project management considerations include adopting the appropriate framework for an ongoing program (as distinct from the rapid response approach of phase 1), consider the sustainability of the project (personnel to meet workload, resources, etc), support and funding, communication, data and information management, media and public engagement and the value of external and internal reviews.



a) b) c)





d) e) f)



g) h) i)

Selected images a) Trapping for Upper Warren Fauna Monitoring and Population Comparison Study; b) checking breeding status as part of the demographics study; c) adult woylie ready for release; d) forensics of woylie body as part of survival and mortality study; e-f) hypogeal fungi (truffles) and spores as part of resources study; g) field health check and disease sampling; h) predator activity surveys using sandpads; i) fox foot print from sandpad surveys.

1.7. References

Abbott, I. 2006. Mammalian faunal collapse in Western Australia, 1875-1925: the hypothesised role of epizootic disease and a conceptual model of its origin, introduction, transmission, and spread. Australian Zoologist **33**:530-561.

Burbidge, A. A., and N. L. McKenzie. 1989. Patterns in the modern decline of Western Australia's vertebrate fauna: causes and conservation implications. Biological Conservation **50**:143-198.

Caughley, G. 1994. Directions in Conservation Biology. Journal of Animal Ecology **63**:215-244.

Freegard, C. 2007. Nomination of a Western Australian species for listing as threatened, change of status or delisting: woylie. Report. Department of Environment and Conservation, Kensington.

Orell, P. 2004. Fauna monitoring and staff training: Western Shield review - February 2003. Conservation Science Western Australia 5:51-95.

Start, A. N., A. A. Burbidge, and D. Armstrong. 1995. Woylie recovery plan. Wildlife Management Program No.16. Western Australian Department of Conservation and Land Management and South Australian Department of Environment and Natural Resources, Perth.

Start, A. N., A. A. Burbidge, and D. Armstrong. 1998. A review of the conservation status of the woylie (Bettongia penicillata ogilbyi (Marsupialia: Pootoroidae) using IUCN criteria. CALMScience **2(4)**:277-289.

Wayne, A. F. 2006. Interim assessment of the evidence for a recent decline in woylie abundance in south-western Australia. Pages 1-28. CALM, Perth, Western Australia.

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CHAPTER 1. INTRODUCTION

Adrian Wayne

Department of Environment and Conservation

Abstract

The history and recent changes in abundance and distribution of woylies is briefly summarised. The principal aim of the woylie conservation research project (WCRP) is to determine the causal factors responsible for the recent woylie declines in southwestern Australia. Using a diagnosis framework based on the 'declining population paradigm' (*sensu lato* Caughley, 1994) and a classification of possible agents of decline into resources, predators, disease and direct human interference, the WCRP focussed on determining the causes in the Upper Warren region, east of Manjimup. The WCRP has been organised into three major components; 1) Upper Warren Fauna Monitoring 2), Meta-analysis, and 3) Population Comparison Study (PCS). Effective and extensive collaborations with numerous agencies and experts have been critical to the effectiveness of this project. The nature of these collaborations, the project management framework, and project milestones are very briefly summarised. The context and purpose if this report is discussed.

1.1. Situation summary of recent woylie population declines

The woylie (*Bettongia penicillata ogilbyi*) had a distribution across much of Australia prior to settlement by Europeans (Figure 1.1). By the 1960's the woylie was reduced to three isolated remnant populations in southwestern Australia (Upper Warren [principally Perup], Tutanning and Dryandra) (Figure 1.2). Fox control and woylie reintroductions began in the 1970's. Since 1996, these activities have been expanded and strategically managed as part of the '*Western Shield*' conservation program (Orell, 2004). These efforts resulted in a dramatic recovery that culminated in the woylie being the first Australian mammal to have its conservation status downgraded as a result of it being delisted from Commonwealth and State conservation lists (Endangered / Threatened) in 1996 (Start *et al.,* 1998).

Since 2001, however, woylie populations throughout southwestern Australia have undergone rapid and substantial declines. For example, capture rates from long-term monitoring and research has indicated that the woylie population at Dryandra declined by 93% between 2000 and 2006. The Upper Warren population(s) underwent a median decline of 95% between 2002 and 2007. The woylie population at Batalling (a 1982 reintroduction site east of Collie; Figure 1.2) declined by 97% between 2002 and 2007. Evidence from multiple sources including live trapping, sandpad surveys, woylie nest and digging densities and spotlight surveys all concur and demonstrate that these declines are real (Wayne, 2006). A concurrent woylie decline has also been observed in South Australia (Venus Bay Peninsula); while South Australian island populations appear to have remained relatively stable (Jason VanWeenan, pers. comm.).

In general, the largest and most substantial woylie populations have undergone substantial declines while the very small and/or low density, often isolated populations appear to be less affected. Overall, an estimated 70-80% decline in woylie numbers has occurred in Australia over five years. These declines are still continuing and there is little evidence of signs of a recovery (Freegard, 2007). Introduced predators (fox and cat) and habitat loss were among the principal factors thought responsible for historical declines (Burbidge and McKenzie, 1989; Start *et al.,* 1995). There also is some anecdotal evidence that disease may have caused the decline of many mammal species (including the woylie) in Western Australia in the late 1800s to early 1900s (Abbott, 2006). The cause(s) of these current declines is unknown.

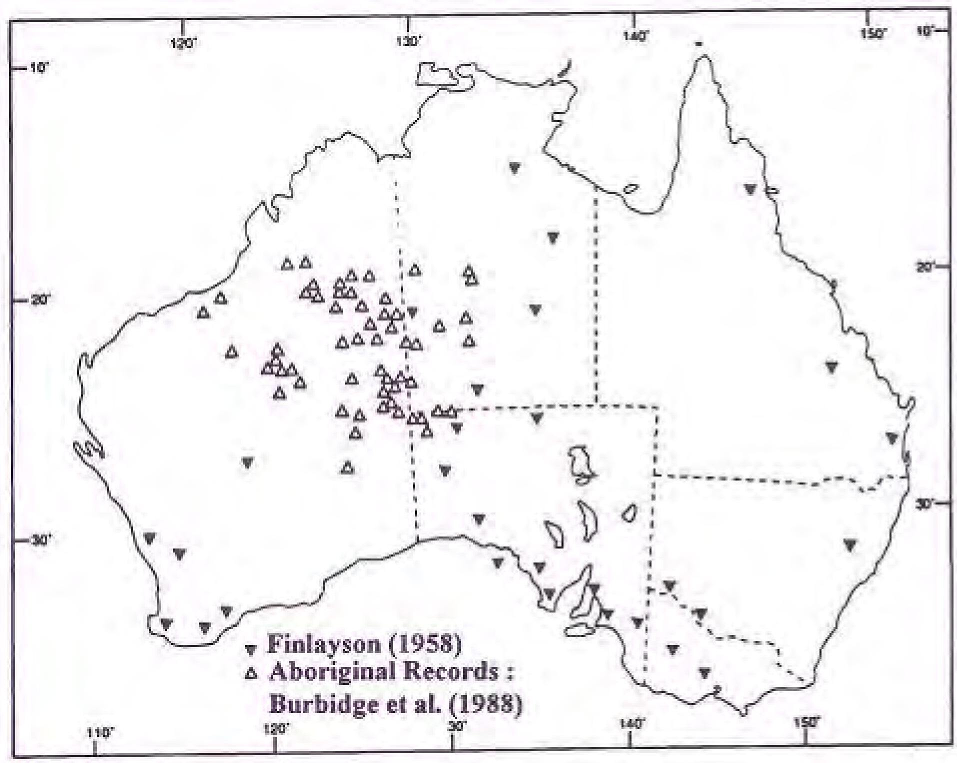

Figure 1
Historic Distribution

Figure 1.1. Historic distribution of woylies (*Bettongia penicillata*). Source: Start *et al.* (1995).

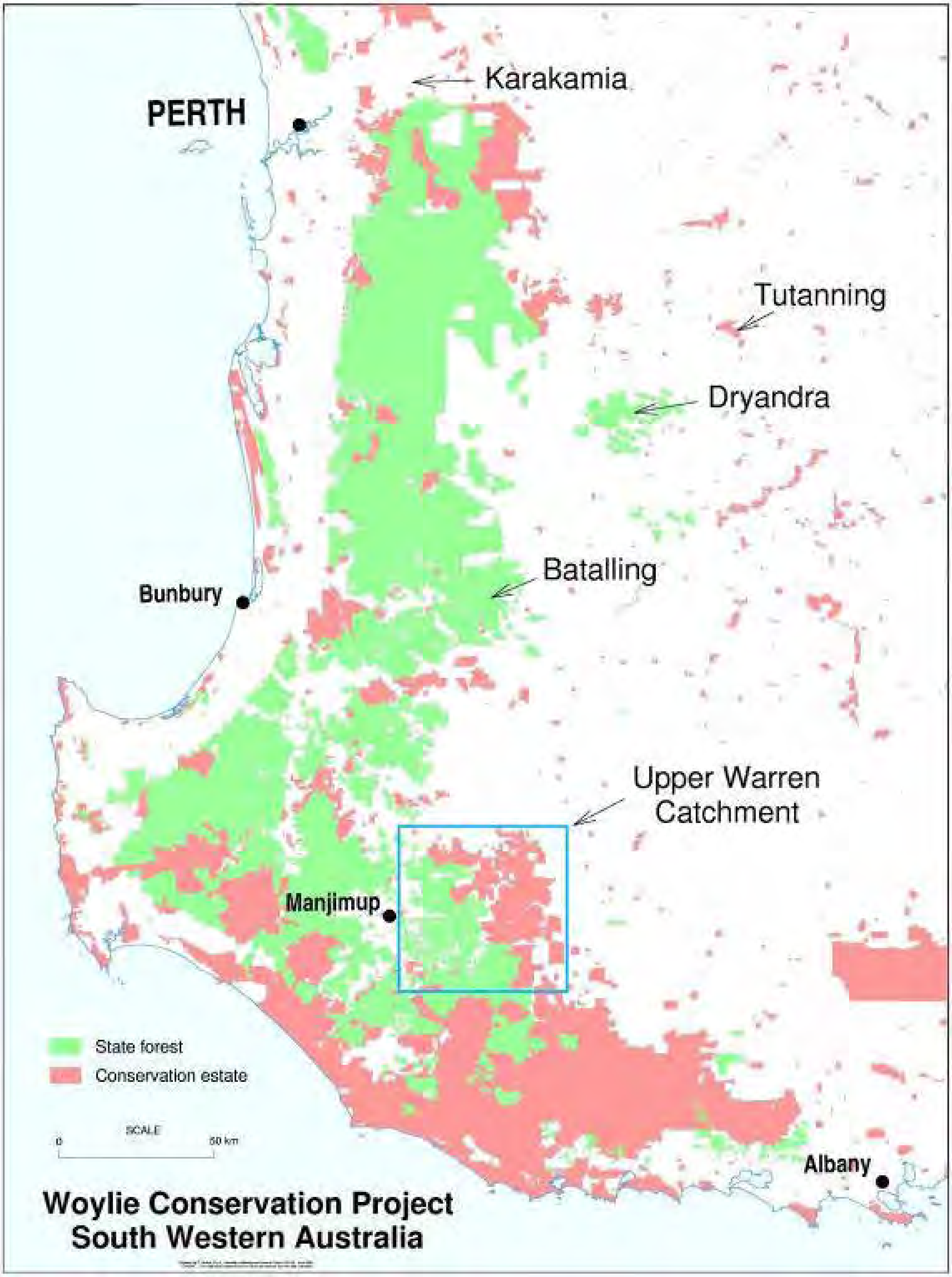

Figure 1.2. The location of important woylie populations.

1.2. The research approach to diagnosing recent woylie declines.

The Department of Environment and Conservation (DEC) has established a comprehensive and collaborative project to diagnose the cause(s) of recent woylie declines.

The principal aims of the woylie conservation research project (WCRP) are;

a) Determine the causal factors responsible for the recent woylie declines in southwestern Australia;

b) Identify the management required to ameliorate these declines; and,

c) Develop mammal monitoring protocols that will better inform factors associated with future changes in population abundances.

Determining the cause(s) of population or species decline is notoriously difficult (e.g. Caughley, 1994; Caughley and Gunn, 1996; Peery *et al.,* 2004). Common challenges identified in the literature include;

- i) Overcoming the complexity due to the likelihood of multiple factors being involved either simultaneously or sequentially.
- ii) The need to separate independent effects to avoid the confounding between factors.
- iii) Discriminating between causes, effects, and associations (coincidental or otherwise).
- iv) Detecting reduced survival or productivity caused by environmental contaminants or disease is rarely straightforward.
- v) The influence of habitat upon the decline of a species is particularly difficult to diagnose and that a safer preliminary hypothesis would conjecture that a species ends up, not in the habitat most favourable to it, but in the habitat least favourable to the agent of decline.
- vi) The need to reduce some causal agents, such as habitat modification, down to the individual processes and specific effects (e.g. resource elements such as food and shelter).
- vii) Avoiding the seduction of the obvious and the easy to measure. Not all agents are so conspicuous, which in no way lessens their importance.

Scientific rigour is, therefore, critical to the success of endeavours to identify the cause(s) of a species' decline. Based on the 'declining population paradigm' and related scientific approaches (e.g. Caughley, 1994; Caughley and Gunn, 1996; Peery *et al.,* 2004) the diagnosis framework used to investigate the recent woylie declines is;

1. Confirm that the population has declined.
2. Determine the spatial, temporal and demographic characteristics of the observed decline.
3. Understand the species' ecology.
4. Identify all potential causes.
5. Use circumstantial evidence to help shortlist the potential causes.
6. Seek direct evidence – test putative causes.
7. Given the evidence, determine the most appropriate conservation and management responses within an active adaptive management framework.

The WCRP uses a hypothetico-deductive approach (as recommended by Caughley, 1994) involving parallel lines of enquiry addressing the numerous possible agents of decline, most of which can be broadly classified into four major groups;

1. Resources – including food depletion and consequences of climate change, fire management, etc.
2. Predation – including native and introduced species, and effectiveness of current control measures.
3. Disease – including known and novel agents (viral, haemaparasites, endoparasites, ectoparasites, bacterial diseases, nutrition and toxicology).
4. Direct human interference – e.g. negative consequences of trapping (over-harvesting for translocations, disrupted breeding success, reduced condition, injuries, increased stress and susceptibility to other mortality factors).

The research has a specific focus on the Upper Warren region to concentrate existing resources in the one area where declines are current. Such a focus is expected to improve the chances of success and eliminate the potential confounding of differences in the factors being potentially at play elsewhere. Nonetheless, information from other woylie populations has been incorporated through collaborations with other research and monitoring wherever appropriate and possible.

The project has three major components that together, address the above diagnosis framework.

1) Upper Warren Fauna Monitoring – an enhancement and co-ordination of existing monitoring and research activities – that provides;

- six-monthly information up-dates on population change and associated characteristics at the regional scale and,
- a regional-scale means of collecting data on woylies and putative agents of decline to complement the finer-scaled population comparison study.

2) Meta-analysis of existing data sets to;

- Confirm that the declines are real
- Quantify the spatial, temporal and demographic characteristics of the woylie decline, which in turn will,
- Provide circumstantial evidence that will aid in the identification of the possible causes of decline.

3) Population Comparison Study (PCS) is a detailed investigation of woylies and the possible agents of decline. This principally involves six sites that support populations at different stages of decline;

- Declined populations now at low densities: Boyicup and Winnejup (Upper Warren Region)
- The last remaining moderate-density woylie populations in the Upper Warren region: Keninup, Warrup and Balban
- High-density and stable population - Karakamia Wildlife Sanctuary (50 km east of Perth), a fenced (i.e. closed) population

The five main lines of enquiry to be investigated at the population comparison study sites are;

- Woylie density and demographics
- Woylie survival and mortality
- Predators
- Resources
- Disease

More detail on the research approach and methodology is provided in the DEC Science Project Proposal (SPP 2007/02).

1.3. The collaborative approach to diagnosing recent woylie declines.

Collaborations with high-quality specialist expertise are considered critical to the successful diagnosis of the recent woylie declines. A large number of close collaborations are particularly necessary to adequately and comprehensively address the complexity, size and breadth of the WCRP. The success and progress of the project is fundamentally a direct result of the commitment and quality of the collaborators and their expertise. The willingness and co-operation of the individuals and institutions involved in this project (Table 1.1) have been exceptional and should be highly commended for their efforts.

Principal collaborating institutions include Department of Environment and Conservation, Murdoch University, Perth Zoo, and Australian Wildlife Conservancy. Collaborations also exist with individuals from the South Australian Government Department of Environment and Heritage, University of Western Australia, Manjimup Aeroclub, Data Analysis Australia (consultants), and University of Adelaide. Over 85 individuals are involved in some ongoing manner with the WCRP (Table 1.1). Many others have been involved on a less frequent or less regular basis. Contributions by volunteers have also been extremely important, particularly in increasing the capacity for data and sample collection in the field. More than 123 individuals have collectively contributed more than 498 days and 3955 hours of volunteer service to the project between January 2006 and July 2007.

1.4. Project management

The management of the logistics, planning, co-ordination and communication required for this project have been extensive, particularly given the inherent complexities of the problem and research, and the number and diversity of the people involved. Figure 1.3.1 provides a summary of the WCRP management structure. The principal roles and responsibilities for the project are summarised in Table 1.2. The details of the project management are not provided here given that this report is focussed on providing an update regarding the development of results.

1.5. Research progress

Key milestones for research into the recent woylie declines include;

Oct 2005	Neil Burrows alerted to possible woylie declines. Adrian Wayne assigned to investigate and verify.
Dec 2005	Completed the survey of 12 transects throughout the Upper Warren region to substantiate and assess the extent and magnitude of woylie declines (i.e. established what became the biannual Upper Warren Fauna Monitoring program).
Feb 2006	Recent mammal declines workshop (DEC internal) convened to examine preliminary findings confirming extensive and substantial woylie declines, within the context of other native species, possible causes and developing response priorities and strategies.
Feb 2006	Establishment of the Woylie Conservation Steering Group to facilitate and assist in the co-ordination of a management and research response to recent woylie declines.
April 2006	Establishment of the Woylie Disease Reference Council to provide expert advice and research assistance into the possible role(s) of disease in recent woylie declines.
May 2006	Submission of situation report and research proposal to CALM Corporate Executive.
July 2006	Commencement of the Woylie Conservation Research Project (BCI funding secured for 2006/07).
May 2007	Completion of the database aggregation of multiple isolated datasets involving DEC fauna trapping in the Upper Warren.
August 2007	Completion of field work (excluding ongoing PhD research programs and some ongoing Upper Warren Fauna Monitoring).

Table 1.1. Summary of collaborators directly involved in the Woylie Conservation Research Project in an ongoing manner.

WCRP Component	Name	Principle WCRP Role	Affiliation
Core WCRP	Ms Marika Maxwell	Predator / Resource Co-ordinator	DEC, Science Div.
Core WCRP	Ms Julia Wayne	Upper Warren Fauna Monitoring Co-ordinator	DEC, Donnelly District
Core WCRP	Ms Sheryn Prior	Data management / Volunteer co-ordination	DEC, Science Div.
Core WCRP	Ms Wendy Sicard	Resources / Technical Support (Exchange)	U.S.A. B.L.M.
Core WCRP	Ms Marnie Swinburn	Disease Deputy Co-ordinator	DEC, Warren Region
Core WCRP	Mr Chris Vellios	Demographics / Resources Deputy Co-ordinator	DEC, Science Div.
Core WCRP	Mr Bruce Ward	Survival Deputy Co-ordinator	DEC, Science Div.
Core WCRP	Mr Colin Ward	Demographics / Survival Co-ordinator	DEC, Science Div.
Core WCRP	Dr Adrian Wayne	Project Co-ordinator / Chief Investigator / Disease Co-ordinator	DEC, Science Div.
Core WCRP	Mr Ian Wilson	District Liaison / UW Fauna Monitoring Co-Coordinator	DEC, Donnelly District
Disease (WDRC)	Mr Yazid Abdad	Parasitology and Microbiology - PhD Student	Murdoch University
Disease (WDRC)	Dr Peter Adams	Parasitology and Microbiology	Murdoch University
Disease (WDRC)	Dr Halina Burmej	Parasitology and Microbiology - PhD Student	Murdoch University
Disease (WDRC)	A/Prof Phil Clark	Haematology	Murdoch University
Disease (WDRC)	Dr Paul Eden	WDRC Clinical Co-ordinator	Perth Zoo
Disease (WDRC)	Dr Trevor Ellis	Viruses	Murdoch University
Disease (WDRC)	A/Prof Stan Fenwick	Microbiology	Murdoch University
Disease (WDRC)	Dr Lisa Hulme-Moir	Clinical Pathology - PhD student	Murdoch University
Disease (WDRC)	Dr Graeme Knowles	WDRC Pathology Co-ordinator	Murdoch University
Disease (WDRC)	A/Prof Alan Lymbery	Parasitology and Ecology	Murdoch University
Disease (WDRC)	Dr Cree Monaghan	Wildlife Clinician and Nutritionalist	Perth Zoo
Disease (WDRC)	Dr Phil Nicholls	Pathology	Murdoch University
Disease (WDRC)	Dr Carlo Pacioni	Genetics and Epidemiology - PhD Student	Murdoch University
Disease (WDRC)	Dr Nevi Parameswaran	*Toxoplasma* - PhD Student	Murdoch University
Disease (WDRC)	Ms Unaiza Parkar	Enteroparasites - PhD Student	Murdoch University

Disease (WDRC)	Dr Andrea Reiss	Wildlife Clinician	Perth Zoo
Disease (WDRC)	A/Prof Ian Robertson	Epidemiology	Murdoch University
Disease (WDRC)	Dr Andy Smith	Parasitology and Ecology (ectoparasites)	Murdoch University
Disease (WDRC)	Prof Andrew Thompson	Principal Murdoch liaison / WDRC co-ordinator	Murdoch University
Disease Support	Mr Gary Allen	Clinical Pathology (Haematology)	Murdoch University
Disease Support	Ms Susana Averis	Haemaparasites	Murdoch University
Disease Support	Ms Ailleen Elliott	Enteroparasites	Murdoch University
Disease Support	Mr Russ Hobbs	Enteroparasites	Murdoch University
Disease Support	Dr Sandy McLachlan	Pathology	Murdoch University
Disease Support	Dr Karen Payne	Wildlife Clinician	Perth Zoo
Disease Support	Dr Shane Raidal	Pathology	Murdoch University
Disease Support	Ms Judy Robertson	Clinical Pathology (Haematology)	Murdoch University
Disease Support	Dr Peter Spencer	C. Pacioni PhD Supervisor	Murdoch University
Disease Support	Dr Kris Warren	C. Pacioni PhD Supervisor	Murdoch University
External Programs	Dr Dave Algar	Mesopredator Project - Mt Gibson	DEC, Science Div.
External Programs	Mr David Armstrong	South Australian Woylie Populations	DEH, SA
External Programs	Mr Paul de Tores	Mesopredator Project - Northern Jarrah Forest	DEC, Science Div.
External Programs	Ms Christina Gilbert	Wellington District - Batalling	DEC, Wellington District
External Programs	Ms Fiona Kirkpatrick	Wellington District - Batalling	DEC, Wellington District
External Programs	Dr Nicki Marlow	Mesopredator Project - Dryandra/Tutanning	DEC, Science Div.
External Programs	Mr Jeff Richardson	Wheatbelt	DEC, Wheatbelt Region
External Programs	Mr Neil Thomas	Mesopredator Project - Dryandra/Tutanning	DEC, Science Div.
External Programs	Mr Jason van Weenen	South Australian Liaison	DEH, SA
External Programs	Mr Andy Williams	Mesopredator Project - Dryandra/Tutanning	DEC, Science Div.
Field Operative	Mr Dave Butcher	Animal Handler	DEC, Donnelly District
Field Operative	Ms Zoe Clarke	Animal Handler	DEC, Donnelly District
Field Operative	Mr Peter Davis	Aerial radio-telemetry Pilot	Manjimup Aeroclub
Field Operative	Mr Jamie Flett	Aerial radio-telemetry	DEC, Science Div.
Field Operative	Mr Wayne Fox	Animal Handler	DEC, Donnelly District
Field Operative	Mr Graeme Liddelow	Predator Deputy Co-ordinator	DEC, Science Div.
Field Operative	Mr Peter McGinty	Aerial radio-telemetry Pilot	Manjimup Aeroclub

Field Operative	Mr Brian Whittred	Animal Handler	DEC, Donnelly District
Forensics	Dr Oliver Berry	DNA forensics (predators)	University of WA
Forensics	Dr Ian Dadour	Forensics	University of WA
Forensics	Ms Denice Higgins	Forensic Odontology	University of Adelaide
Forensics	Mr Chris O'Brien	Forensics	University of WA
Meta-analysis	Dr John Henstridge	Statistical analysis consultancy	Data Analysis Australia
Meta-analysis	Mr David McKenzie	Database consultancy	Private Consultant
Meta-analysis	Ms Anna Munday	Statistical analysis consultancy	Data Analysis Australia
Meta-analysis	Mr Matthew Williams	Biometrical advice and support	DEC, Science Div.
Meta-analysis	Ms Christine Freegard	Woylie conservation status review, *Western Shield*	DEC, Nature Cons.
Miscellaneous	Ms Leslie Harrison	Principal Wildlife Carer	Volunteer
PCS	Ms Alison Dugand	Karakamia	AWC
PCS	Ms Trish Gardner	Karakamia	AWC
PCS	Dr Jacqui Richards	AWC Liaison	AWC
PCS	Ms Jo Williams	Karakamia Co-ordinator	AWC
Project Management	Ms Robyn Bowles	Administrative Support	DEC, Science Div.
Project Management	Ms Lauren Daubney	Data entry	DEC, Donnelly District
Project Management	Ms Janine East	Volunteer Co-ordinator	DEC, Donnelly District
Project Management	Mr Keith Morris	WDRC, WSORC, FCPL, WCSG, Mesopredator Project	DEC, Science Div.
Project Management	Ms Katie Schoch	Media liaison	DEC, Corporate Affairs
Resources	Dr Neil Bougher	Mycology	DEC, Science Div.
Resources	Dr Kate Bryant	K. Rodda PhD Supervisor	Murdoch University
Resources	Dr Mike Calver	K. Rodda PhD Supervisor	Murdoch University
Resources	Ms Julie Fielder	Mycology	DEC, Science Div.
Resources	Dr Richard Robinson	Mycology	DEC, Science Div.
Resources	Ms Kerry Rodda	Diet and food availability - PhD Student	Murdoch University
Resources	Dr Barbara Wilson	K. Rodda PhD Supervisor	Murdoch University
WCSG	Mr Brad Barton	WCSG Chair	DEC, Warren Region
WCSG	Mr John Gillard	Donnelly District Manager	DEC, Donnelly District
WCSG	Mr Nigel Higgs	Media Liaison	DEC, Corporate Affairs
WCSG	Mr Peter Orell	Database development, *Western Shield*, Meta-analysis, WSORC	DEC, Nature Cons.

WCRP = Woylie Conservation Research Project

WDRC = Woylie Disease Reference Council

WCSG = Woylie Conservation Steering Group

AWC = Australian Wildlife Conservancy

USA BLM = USA Bureau of Land Management

Note: This summary list may not be comprehensive. The inadvertent omission of principal collaborators is an unintentional oversight for which sincere apologies are given in advance.

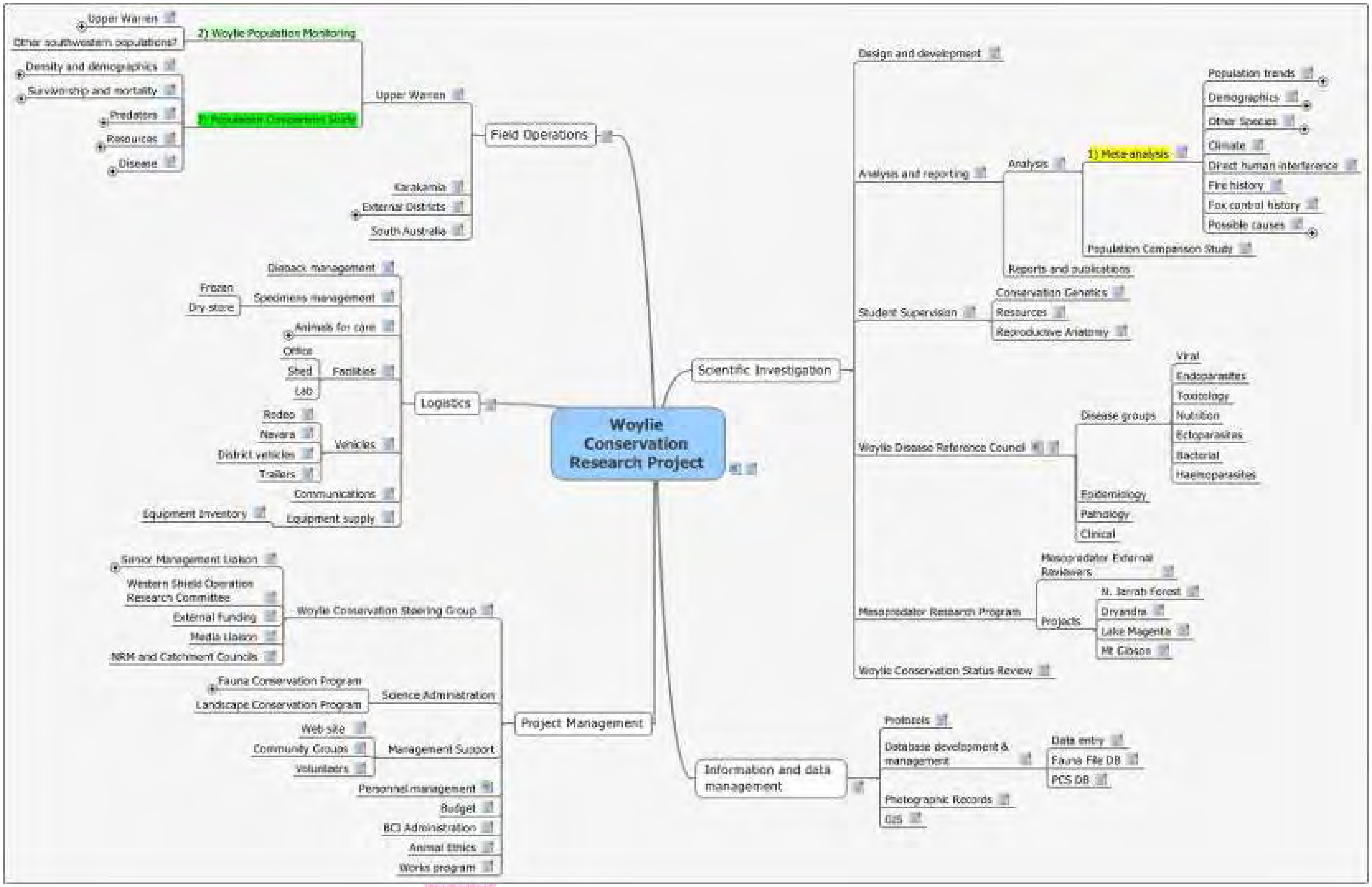

Figure 1.3. Summary of the Woylie Conservation Research Project management structure and components

Table 1.2. Summary of principal Woylie Conservation Research Project roles and responsibilities.

WCRP Component	Operations Leader	Deputy
Project Manager/Chief Investigator	Adrian Wayne	Keith Morris / Lachlan McCaw
1) Upper Warren Fauna Monitoring	Ian Wilson	Julia Wayne
2) Meta-analysis	Adrian Wayne	Matthew Williams
3) Population Comparison Study		
a) Demographics	Colin Ward	Chris Vellios
b) Survival and Mortality	Colin Ward	Bruce Ward
c) Predators	Marika Maxwell	Brian Whittred
d) Resources	Kerry Rodda / Marika Maxwell	Adrian Wayne
e) Disease	Adrian Wayne	Marnie Swinburn
WDRC Chair	Andrew Thompson	Adrian Wayne
WDRC Clinical Co-ordination	Paul Eden	
WDRC Pathology Co-ordination	Graeme Knowles	
Woylie Conservation Steering Group	Brad Barton	Adrian Wayne
Donnelly District Liaison	Ian Wilson	
Karakamia Liaison	Jo Williams	
South Australia Liaison	Jason van Weenen	
External Programs Liaison	Marnie Swinburn	
Field Logistics	Chris Vellios	
Information and Data Management	Marika Maxwell	Peter Orell / Sheryn Prior

1.6. Report outline

The purpose of this report is to collate the results from progress achieved to date across the many components of the WCRP. As such, this report provides an opportunity to examine the accumulating evidence that will assist in the diagnosis of recent woylie declines. On all accounts the results presented here are preliminary. The intent is to provide a timely update on progress that will enable some anticipation as to where the evidence may be leading the diagnosis of woylie declines, which may allow the initiation of planning and preparation of subsequent responses to the current woylie situation. It is premature to draw definitive conclusions on the basis of information provided here given that much of what is reported is descriptive and awaits more rigorous statistical and scientific testing. Nonetheless, this report will inform all those involved in the project, interested managers and DEC Corporate Executive of most of the initial findings. It will also help guide the refinement of analyses of existing datasets and begin the formulation of response plans beyond the current investigative phase.

The structure of the report reflects the structure of the project. The authors of each section generally include many of the principal individuals directly involved in that component of the project. Most of the sections and their content can be regarded as precursors to planned publications in the peer-reviewed literature.

1.7. References

Abbott, I. 2006. Mammalian faunal collapse in Western Australia, 1875-1925: the hypothesised role of epizootic disease and a conceptual model of its origin, introduction, transmission, and spread. Australian Zoologist **33**:530-561.

Burbidge, A. A., and N. L. McKenzie. 1989. Patterns in the modern decline of Western Australia's vertebrate fauna: causes and conservation implications. Biological Conservation **50**:143-198.

Caughley, G. 1994. Directions in Conservation Biology. Journal of Animal Ecology **63**:215-244.

Caughley, G. C., and A. Gunn 1996. Conservation Biology in Theory and Practice. Blackwell Science, Cambridge.

Freegard, C. 2007. Nomination of a Western Australian species for listing as threatened, change of status or delisting: woylie. Report. Department of Environment and Conservation, Kensington.

Orell, P. 2004. Fauna monitoring and staff training: Western Shield review - February 2003. Conservation Science Western Australia 5:51-95.

Peery, M. Z., S. R. Beissinger, S. H. Newman, E. B. Burkett, and T. D. Williams. 2004. Applying the declining population paradigm: Diagnosing causes of poor reproduction in the marbled murrelet. Conservation Biology **18**:1088-1098.

Start, A. N., A. A. Burbidge, and D. Armstrong. 1995. Woylie recovery plan. Wildlife Management Program No.16. Western Australian Department of Conservation and Land Management and South Australian Department of Environment and Natural Resources, Perth.

Start, A. N., A. A. Burbidge, and D. Armstrong. 1998. A review of the conservation status of the woylie (Bettongia penicillata ogilbyi (Marsupialia: Pootoroidae) using IUCN criteria. CALMScience **2(4)**:277-289.

Wayne, A. F. 2006. Interim assessment of the evidence for a recent decline in woylie abundance in south-western Australia. Pages 1-28. CALM, Perth, Western Australia.

CHAPTER 2 UPPER WARREN FAUNA MONITORING

Julia Wayne[1], Adrian Wayne[1], Ian Wilson[1]

Department of Environment and Conservation

Abstract

The Upper Warren Fauna Monitoring component of the Woylie Conservation Research Project comprised biannual monitoring of 11 key transects within the Upper Warren area. Detailed demographic data was collected for all mammal species caught, with the aim of monitoring population change across the region. In addition, detailed health checks of all woylie individuals were conducted and a variety of samples were collected from all mammal species to be used for analysis of health, disease, diet and genetics. Of the eleven transects ten have shown significant recent decline. It remains uncertain when the declines began in some areas due to a lack of data between 1998 and 2005. For areas within the Upper Warren where there is sufficient data, declines appear to have started from 2002 – 2006 and are still continuing. By 2007, the Upper Warren transect populations had undergone a 95% median decline. Nine out of 11 transects currently have 0-8% capture rates (i.e. 0-15% of their pre-decline capture rates). Woylies currently persist at high capture rates at only one transect (Keninup), which has not yet undergone a contemporary decline. The remaining transect (Warrup) currently supports moderate capture rates but appears to have undergone a decline whilst monitoring was not undertaken in this area, between 1998 and 2001. Continued monitoring of the latter two transects, in particular, on a biannual basis is recommended in order to detect the beginning of any decline in a timely manner to enable greatest extraction of information.

2.1. Introduction

Cage trapping was conducted on a biannual basis along 11 transects in the Upper Warren area (Figure 2.1, Table 2.1) to monitor changes in woylie populations including abundance and demographics. Some key potential agents of population change were also monitored in association, where possible. These have been addressed in other sections within this report and include predators, food resources, and disease. This program has built on the monitoring and research activities conducted over the past 30 years to develop a longitudinal context to population changes.

Of the 11 transects, eight were routinely monitored by existing District, Science and training programs prior to the commencement of this project. Two transects were monitored on a biannual basis and the rest on an annual basis. In October 2005, three additional transects were established (one re-established, one extended and one new transect; Winnejup, Keninup2 and Corbal, respectively) to provide a more complete spatial assessment of woylie population changes within the region.

For this project, the monitoring of these 11 transects had:- expanded to biannual surveys, standardised methodology through development of monitoring protocols, become centrally coordinated and the timing of surveys synchronised.

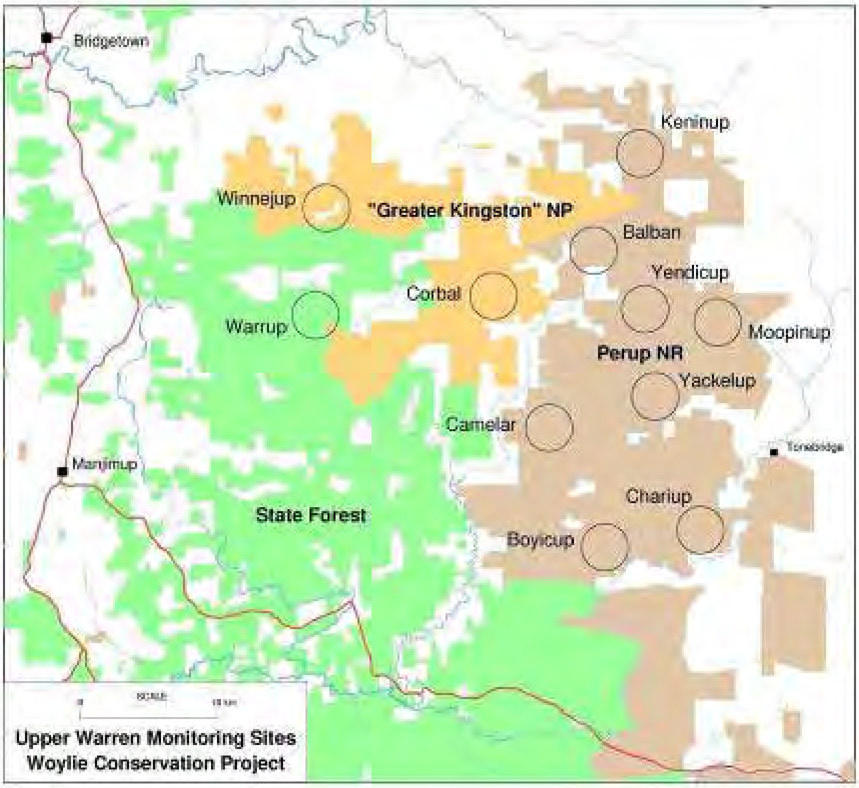

Figure 2.1. Upper Warren Fauna Monitoring sites.

Table 2.1. Locations, ownership and purpose of the transects comprising the Upper Warren Fauna Monitoring.

TRANSECT	LOCATION	AREA	OWNERSHIP	ORIGINAL PURPOSE
Keninup2	Keninup block west of Distributer Rd and North of Westbourne Rd. Transect extended for Woylie Conservation Project	Perup North	Donnelly District	Educational – Perup Ecology Centre.
Balban	Balban block – Numbat Rd	Perup North	Species and Communities Branch	Fauna Management Course Training
Moopinup	Moopinup block – Possum Rd and Stretch Rd	Perup Central	Donnelly District	Western Shield monitoring
Yendicup2	Yendicup block – Bandicoot Rd	Perup Central	Science Division	Bushranger and Long-term monitoring
Yackelup	Yackelup block – Old Boyup Cranbrook, Quenda and Mardoo Rds.	Perup Central	Science Division	Bushranger and Long-term monitoring
Camelar	Camelar block – Fordson and Camelarup Rds.	Perup Central	Species and Communities Branch	Fauna Management Course Training
Chariup	Chariup block – Mordalup creek system south of DeLangraaft Rd	Perup South	Donnelly District	Fire Effects Monitoring
Boyicup2	Boyicup block – Boyicup Rd and Orient Rd.	Perup South	Donnelly District	Western Shield Monitoring
Winnejup	Winnejup block – North, Walcott and Boundary Rds (subset of Kingston Study)	Perup South	Science Division	Kingston Study
Corbal	Corbal block – Eclipse, Morrison and Argument Rds.	Greater Kingston	Science Division	Woylie Conservation Project
Warrup2	Warrup and Kingston blocks – Warrup, Seaton Ross, Maisey and Wilson Rd (subset of Kingston Study)	Greater Kingston	Donnelly District	Western Shield Monitoring (Previously Kingston Study)

2.2. Methods

2.2.1. Upper Warren Fauna Monitoring Methodology

The biannual monitoring was conducted in October/November and March/April of each year (beginning October 2005). Trap setting, bait recipe, trap hygiene, trap clearing, animal processing, sample collection and data scribing were all conducted in accordance with the monitoring and hygiene standards and protocols described in; the Departmental 'Animal Ethics Standard Operating Procedures' (CALM, 2005), the local 'WCRP Operations Handbook (Volume 3)' and the Departmental 'Minimising Disease Risk in Wildlife Management' (Chapman *et al.*, 2005).

Each team had between two and four people depending on the sampling requirements and expected capture rates. The team comprised: an animal handler, a data scribe, a person trained in blood extraction (where required) and an additional assistant (where required). On some of the busier transects two teams were used to process the animals.

Standard processing of animals included:- age, identification tags/microchips, sex, breeding/pouch condition, pouch young biometrics, size and weight measurements for condition indices, parasite loading, body condition rating, coat condition rating, health comments and fate. In addition, full health checks were conducted on all woylies captured and additional sampling was conducted for many individuals. Additional sampling included blood, scats, and ectoparasites for disease screening; scats for dietary analysis (including predators); and ear tissue for DNA analysis. The methodology and results of this sampling has been discussed in Chapter 5 Disease.

2.2.2. WCRP Operations Handbook

An Operations Handbook has been developed through collaboration of Science Division, Donnelly District and Warren Region personnel over the life of the project in an attempt to standardise work procedures and data collection methodologies used during the WCRP fauna monitoring works. The handbook covers such things as data collection, terminology, hygiene, data validation, sample collection and animal and personnel welfare. This handbook was initially used to standardise the monitoring works involved with the WCRP, however it has now been adopted as the standard protocols for fauna monitoring works undertaken within the district and by other projects within the Department. The WCRP Operations Handbook is a working document and continues to undergo review and improvement. It is envisaged that this handbook will form the basis for the development of corporate fauna monitoring protocols. The Handbook will be sent to the *Western Shield* Operations and Research Committee for consideration as the foundation of the new corporate monitoring protocols. The WCRP Operations Handbook in its current form has been distributed for use by other fauna management programs and is being used in part or whole by: Department of Environment and Heritage in South Australia, Paul de Tores in the northern jarrah forest, Nicki Marlow in the wheatbelt and at Lorna Glenn.

See Volume 3 for the complete WCRP Operations Handbook.

2.3. Results

The history of surveys conducted prior to and as part of the WCRP are summarised in Table 2.2. All eleven transects have had four surveys conducted throughout the duration of the WCRP initial field component (October 2005 – June 2007). In addition, six of these transects have been surveyed in October/November 2007. One transect (Corbal) has no historical survey data, the remainder have had between five and 27 previous surveys conducted.

Table 2.2. Surveys conducted on each of the 11 Upper Warren Fauna Monitoring transects prior to and during the project.

TRANSECT	#SURVEYS PRE-2005	OCT/NOV 2005	MAR/APR 2006	OCT/NOV 2006	MAR/APR 2007	OCT/NOV 2007
Keninup2	5*	Y	Y	Y	Y	Y
Balban	6	Y	Y	Y	Y	Y
Moopinup	8	Y	Y	Y	Y	N
Yendicup2	12	Y	Y	Y	Y	Y
Yackelup	12	Y	Y	Y	Y	Y
Camelar	6	Y	Y	Y	Y	Y
Chariup	8	Y	Y	Y	Y	N
Boyicup2	9	Y	Y	Y	Y	N
Winnejup	22	Y	Y	Y	Y	N
Corbal	0	Y	Y	Y	Y	N
Warrup2	27*	Y	Y	Y	Y	Y

Note: * Number of surveys Pre-2005 for Keninup2 and Warrup2 transect include surveys conducted on Keninup1 and Warrup1transects respectively. These are very similar transects within the same area, with slightly different methodologies surveyed by different groups (e.g. slightly different transect locations, trapping frequency, etc)

All six of the monitoring transects within the Perup South and Central areas have shown significant and rapid declines commencing between 2002 and 2004. All have reached very low local population levels in the past two years (Figure 2.2). In the Perup North area, woylie capture rates commenced a rapid decline in 2006 on the Balban transect (76% decline based on surveys using 'smelly' bait or 32% decline based on surveys using 'universal' bait, within a 6-12 month period), yet continue to increase on the Keninup2 transect (Figure 2.3). The increases seen in woylie capture rates on the Keninup2 transect are likely to be, at least in part, an artefact of trap learning by woylies on the newly established transect.

Within the Greater Kingston area (Figure 2.4) the capture rates of woylies on the Warrup2 transect has declined significantly from those recorded during the Kingston Study on Warrup1 transect prior to 1998. Since re-establishment of a subset of the Warrup1 transect (Warrup2) woylie numbers remained fairly stable between 2001 and 2004 and have subsequently increased, coinciding with an increase in survey frequency from annual to biannual (i.e. at least some of this increase is an artefact of changed trapping frequency and trap learning by woylies). The Corbal transect has had a decrease in capture rates of woylies since it's commencement in 2005. The Winnejup transect was not monitored between 1998 and 2005. The capture rates were substantially less in 2005 (when trapping recommenced) compared with the capture rates in 1998. Since re-establishment of monitoring in 2005 there have been increases in capture rates, albeit at low levels.

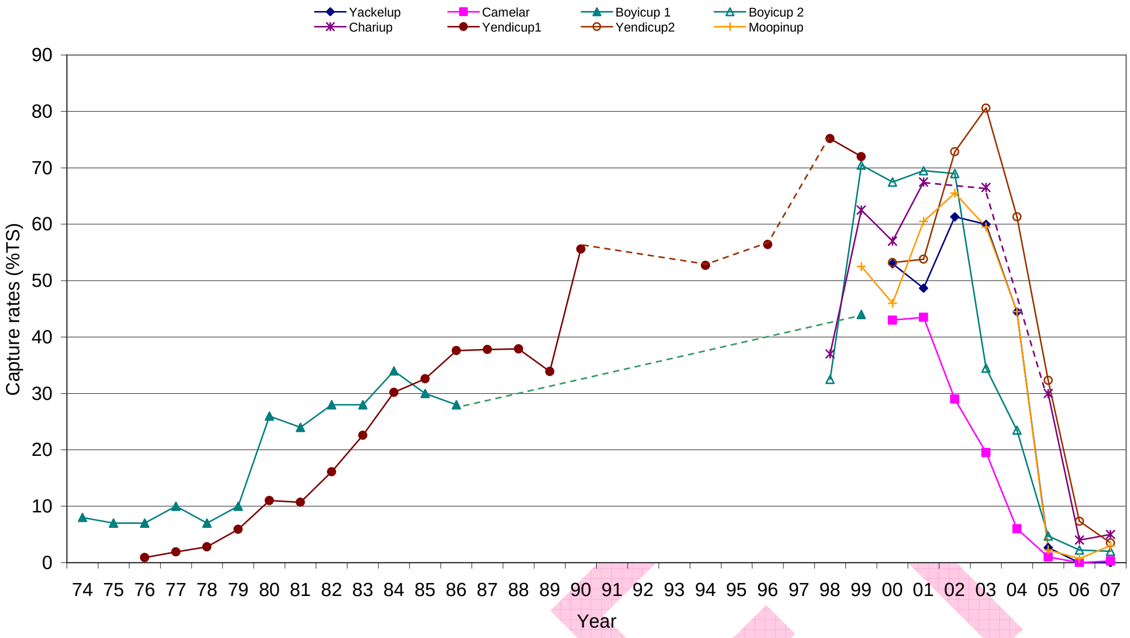

Figure 2.2. Capture rates of woylies over time on each of the Upper Warren Fauna Monitoring transects in southern and central areas of Perup Nature Reserve.

Note: Transect names with the suffix 1 and 2 distinguish relatively similar transects within the same area with slightly different methodologies surveyed by different groups (e.g. slightly different transect locations, trapping frequency, etc).

The dashed lines are indicative trends during the intervening periods between trapping events in non-successive years.

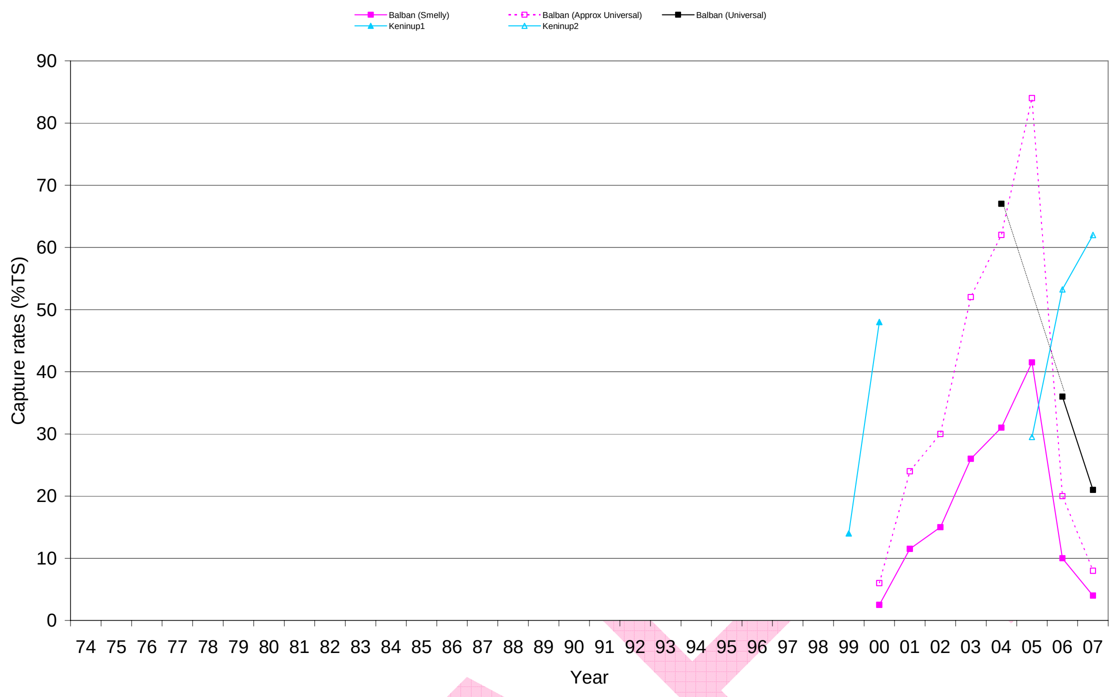

Figure 2.3. Capture rates of woylies over time on each of the Upper Warren Fauna Monitoring transects in the northern areas of Perup Nature Reserve.

Note: Transect names with the suffix 1 and 2 distinguish relatively similar transects within the same area with slightly different methodologies surveyed by different groups (e.g. slightly different transect locations, trapping frequency, etc).

'Balban (Smelly)' is an atypical transect given that it uses a pro-chuditch bait, which catches approximately 50% of the woylies than conventional universal bait.

'Balban (Approx Universal)' is an estimation of woylie numbers that would be expected to be caught on the Balban (Smelly) surveys, had universal bait been used instead of smelly bait (i.e. values = 2 x Balban smelly data)

'Balban (universal)' are surveys on the identical transect as Balban (smelly), the only difference being that universal bait was used throughout the survey rather than smelly bait.

The dashed lines are indicative trends during the intervening periods between trapping events in non-successive years.

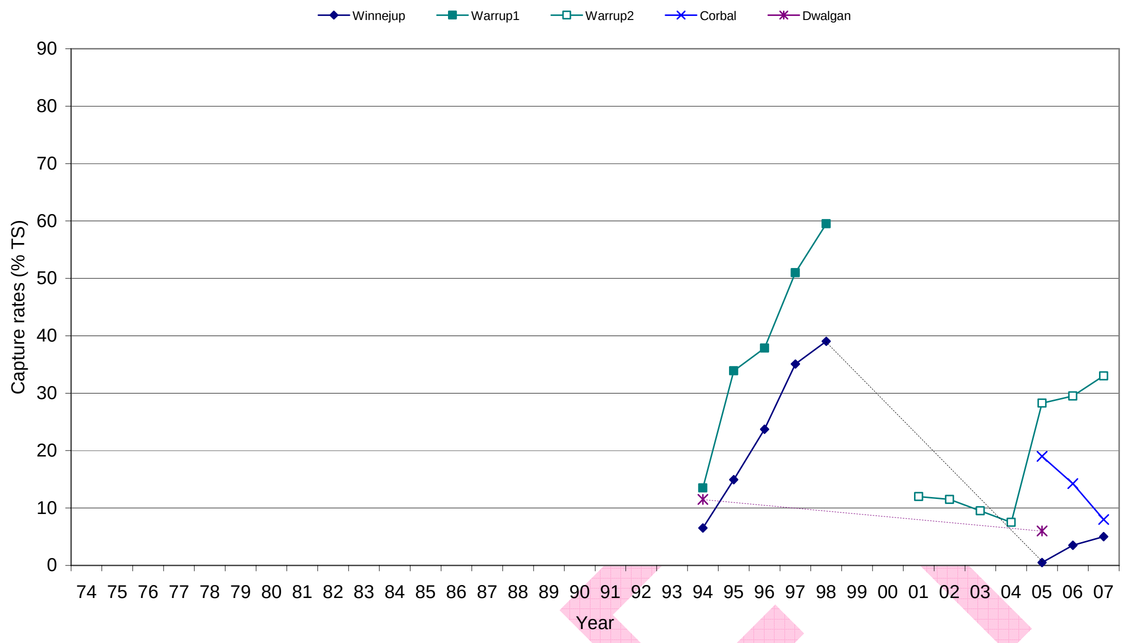

Figure 2.4. Capture rates of woylies over time on each of the Upper Warren Fauna Monitoring transects in the Greater Kingston area.

Note: Transect names with the suffix 1 and 2 distinguish relatively similar transects within the same area with slightly different methodologies surveyed by different groups (e.g. slightly different transect locations, trapping frequency, etc).

The dashed lines are indicative trends during the intervening periods between trapping events in non-successive years

Other data available for the area and from the Kingston study is not presented here due to differences in sampling methodology and confounding with timber harvesting activities making it inappropriate for direct comparison. Despite these differences, data from 1994-2000 and 2004/05 is consistent with the decline patterns observed elsewhere in the Upper Warren.

The pre-decline average for each transect has been calculated using the average of the woylie capture rates (%TS) for the three years immediately preceding the down-turn in the population (where possible). The pre-decline averages for Warrup2 and Winnejup transects were derived from the last 3 years of the Kingston Study monitoring data. The pre-decline average for Balban transect was derived from the 2004 trapping session using universal bait. Pre-decline averages could not be determined for the two newly established transects (Keninup2 and Corbal).

In summary, the mean and median extent of recent woylie decline (based on capture rates) in the Upper Warren has been 86% and 95% respectively (Table 2.3). It should be noted, however, that when considering the mean, the strong leverage of the higher capture rates on the Warrup2 transect should be taken into account. Seven of the nine transects for which there is pre-decline data, have declined by more than 90% of the woylie capture rates in 2007, when compared with their pre-decline average (Table 2.3).

Table 2.3. Summary of capture rates (% TS) of woylies and percentage of pre-decline population extant during 2005-2007 monitoring sessions on each of the 11 Upper Warren Fauna Monitoring transects.

	PRE-DECLINE	NOV 2005		APR 2006		NOV 2006		APR 2007		NOV 2007	
TRANSECT	AVE. %TS	%TS	EXTANT	%TS	EXTANT	%TS	EXTANT	%TS	EXTANT	%TS	EXTANT
Keninup2	-	30	-	51	-	56	-	64	-	59	-
Balban	67	42*	63%	36	54%	20*	30%	21	31%	4*	6%
Moopinup	62	0	0%	0	0%	2	3%	3	5%	-	-
Yendicup2	64	11*	17%	8*	12%	7*	11%	3*	5%	4*	6%
Yackelup	56	1*	2%	0*	0%	0*	0%	0*	0%	0*	0%
Camelar	43	1	2%	0	0%	0	0%	0	0%	1	2%
Chariup	62	22	35%	6	10%	2	3%	5	8%	-	-
Boyicup 2	69	6	9%	3	4%	2	3%	2	3%	-	-
Winnejup	33	1	3%	7	21%	1	3%	5	15%	-	-
Corbal	-	19	-	16	-	13	-	8	-	-	-
Warrup2	50	28	56%	33	66%	27	54%	33	66%	33	66%
NB: Pre-decline average for Balban derived from 2004									Mean (2007)		14%
* Balban, Yendicup and Yackelup - Universal bait only (extrapolated for Balban Nov sessions using smelly bait)									Median (2007)		5%

2.4. Discussion

All eleven of the Upper Warren Fauna Monitoring transects have had at least four monitoring sessions since the commencement of the WCRP in October 2005. All transects were monitored on a biannual basis in October/November and March/April each year during the initial field-based investigative stage of the WCRP (October 2005 to June 2007).

Significant declines in woylie populations (measured by capture rates) have occurred throughout most parts of the Upper Warren region commencing between 2002 and 2006 (Figures 2.2-4). The declines have been greatest in the Perup Central and Perup South areas with an average decline of 96% (Figure 2.2). In this area, three of the six populations have reached undetectable levels during the 2005 to 2007 period. It should be noted, however, that two of these populations have in recent times reached detectable levels once again and may well have commenced a recovery phase. Woylie populations have started declining more recently and continue to decline in some parts of Perup North (Balban) (Figure 2.3).

Three of the eleven transects (Warrup2, Winnejup and Keninup2) have shown differing responses to that of other transects during the period of recent declines, with no decline being observed between 2005 and 2007 (Figures 2.3-4). Increases in woylie capture rates around 2005/2006 on these transects (Figures 2.3-4) coincided with an increase in survey frequency from irregular or annual to biannual. It is expected that at least some of this increase is an artefact of changed trapping frequency and trap learning by woylies.

It should be noted that both Warrup2 and Winnejup transects have shown significant declines in woylie capture rates since earlier monitoring efforts prior to 1999 as part of the Kingston Study. Due to the absence of monitoring data between 1998 and 2001 (Warrup) or 2005 (Winnejup) the timing of the commencement of decline is unknown. In the case of the Warrup transect, it appears that decline commenced earlier than is apparent on other transects in the Upper Warren region. It is unknown whether the decline which has occurred here is related to the current declines being experienced. If in fact this does represent one of the earliest commencements of the current decline, then it is possible that we are now monitoring a population in recovery phase (given the recent population increases).

The changes in woylie population abundance and demographics for each of the 11 Upper Warren Fauna Monitoring transects have been analysed in Chapter 3 Meta-analysis. It should be noted that two of the 11 transects were established in 2005 and therefore have no existing pre-decline data on woylie relative abundance.

2.5. Future work

With the completion of the initial phase of the Woylie Conservation Research Project, monitoring within the Upper Warren region will revert back to the standard monitoring programs conducted within recurrent budgets. This will mean that eight of the 11 transects will continue to be monitored on at least an annual basis.

It is recommended that the two transects (Keninup2 and Warrup2) which remain at highest densities and have yet to show evidence of significant recent decline, continue to be monitored on a biannual basis until the cause of decline has been substantiated. These transects may assist in the identification of the cause(s) of decline by providing supporting data (e.g. morphometrics, health checks, demographics, etc) as well as supporting the collection of samples for analysis (e.g. disease and diet). Through regular monitoring of these transects, a decline if it occurs, may be detected more rapidly allowing the opportunity to maximise the collection of data from individuals at the critical time of decline.

Over the next 12 months (2008), Keninup2, Warrup2, Yackelup and Yendicup2 (as a minimum) will continue to be monitored on a biannual basis. The eight transects which will continue to be monitored as part of ongoing monitoring programs provide representatives of high (H), moderate (M) and low (L) density sites (Table 2.4). These transects also provide representatives of currently declining, declined, stable and (potentially) recovering populations.

The protocols set out in the WCRP Operations Handbook (Volume 3) will continue to guide the monitoring standards and requirements for all future fauna trapping within the District. Additional sampling requirements will be determined by Science Division and within budget constraints.

Table 2.4. Planned monitoring sessions on each of the 11 Upper Warren Fauna Monitoring transects over the next 12 months (2008).

	MONITORING SESSION			
TRANSECT	MAR/APR 2008	OCT/NOV 2008	NO. SESSIONS TO DEC 2008	WOYLIE DENSITY IN 2007
Keninup2	Y	Y	2	H
Balban	N	Y	1	L
Moopinup	Y	N	1	L
Yendicup2	Y	Y	2	L
Yackelup	Y	Y	2	L
Camelar	N	Y	1	L
Chariup	Y	N	1	L
Boyicup2	Y	N	1	L
Winnejup	N	N	0	L
Corbal	N	N	0	L
Warrup2	Y	Y	2	M

2.6. Conclusion

A long history of monitoring data within the Upper Warren region has provided essential data for substantiating the extent of declines in woylie populations. Biannual monitoring of 11 transects spatially distributed throughout the range of the woylie in the Upper Warren region has shown continued declines on most of the transects – particularly within the Perup area. There are a variety of woylie population change patterns across transects including; stable, declining, declined and (potentially) recovering. Representatives of each of these should continue to be monitored on an annual basis as a minimum until the causes and mechanics of decline and recovery are better understood.

At the commencement of this project, the need for more thorough and rigorous protocols for monitoring was realized. During the project such protocols have been developed and these are now used as the standard for monitoring practices within the Donnelly District and beyond. These protocols have been put forward as the basis for a revised Departmental monitoring protocols.

2.7. Acknowledgements

We would like to acknowledge the contributions of Donnelly District, Manjimup Science Division, Warren Region and Wellington District staff and Fauna Management Course organisers and participants in the fieldwork components of the Upper Warren Fauna Monitoring program.

We would also like to acknowledge the large number of volunteers who have put in an enormous amount of time to assist with field works. Without the assistance of these volunteers the extensive and demanding field program would not be possible to achieve.

2.8. References

CALM, 2005. Department of Conservation and Land Management Animal Ethics Standard Operating Procedures. Department of Conservation and Land Management, Perth.

Chapman, T., Sims, C., Mawson, P., 2005. Minimising Disease Risk in Wildlife Management. Standard operating procedures for fauna translocation, monitoring and euthanasia in the field. Department of Conservation and Land Management, Perth.

CHAPTER 3 META-ANALYSIS OF CORPORATE DATA

3.1. Meta-analysis overview

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Meta-analyses of existing medium-sized mammal data from past and present research and monitoring was used to capitalise on the available data to determine and characterise the attributes and associations of woylie population change. This investigative approach was considered an efficient and potentially critically-important means of providing supporting circumstantial and associative evidence that directly assists in the diagnosis of the woylie declines. The meta-analyses at the Upper Warren regional scale have been addressed and are reported here. Meta-analyses at the southwestern Australian scale have not yet been systematically conducted. The framework for the Upper Warren region meta-analysis investigations included;

1) Fauna data aggregation from multiple isolated datasets into a centralised relational database to enable subsequent analyses (Section 3.2).
2) Assessment of whether the woylie capture rates are indicative of real woylie population declines (Volume 2. Appendix 1)
3) Temporal characterisation of the woylie population changes (Section 3.3)
4) Spatial characterisation (preliminary only) of the woylie population changes (Section 3.4)
5) Demographic changes associated with woylie population changes (Section 3.3), including;
   - Sex ratio
   - Age structure
   - Weight
   - Hindfoot length
   - Condition index (biometric algorithm based on size and weight)
   - Proportion of breeding adult females
6) Associative changes in other sympatric fauna. To date, only the medium-sized mammals for which there is readily available and directly comparable trapping data have been investigated (i.e. koomal, *Trichosurus vulpecula*; quenda, *Isoodon obesulus*; and chuditch, *Dasyurus geoffroii*). These represent potentially comparable resource competitors, similarly-sized prey species and a potential native predator of woylies (Section 3.3).
7) Direct human interference - principally whether there are possible population-scale effects of live trapping and live harvesting for translocations (Section 3.5).
8) Fire – i.e. whether woylie declines were associated with fire regime and/or history differences (Section 3.6).
9) Climate – i.e. whether woylie declines throughout the southwest were associated with the primary climate and weather attributes (Section 3.7)

Some of the meta-analyses (temporal, demographic and sympatric fauna associations) were conducted under contract with ‘Data Analysis Australia’ statistical consultants. These components are presented together in Section 3.3.

3.2. Information and data management

Marika Maxwell[1], Adrian Wayne[1], David McKenzie[2], Peter Orell[1], Sheryn Prior[1], Ian Wilson[1], Julia Wayne[1]

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Abstract

The use of existing data relating to fauna in the Upper Warren region to assist in the diagnosis of recent woylie declines was substantially compromised by the problems associated with the data being located on multiple isolated databases, maintained by multiple custodians and in various formats. In response, two closely-linked databases were created to manage the Woylie Conservation Research Project (WCRP) requirements; 1) Manjimup Fauna File (MFF) - an aggregation of existing fauna trapping data common to most datasets from the Upper Warren, and 2) Woylie PCS database to manage the detailed data unique and specific to the WCRP.

The principal datasets that were aggregated to create the MFF included the previously isolated databases associated with the Kingston Project, long-term monitoring of Perup, Bushrangers, *Western Shield*, DEC Fauna Management Course, and Donnelly District fauna activities. The aggregation resulted in 39,125 fauna records being incorporated into the MFF with the 12,000 existing fauna records. Over 25,000 woylie records spanning more than 30 years have subsequently been aggregated and made available for analyses that will assist in the diagnosis of the recent woylie declines.

Woylie PCS is the second, linked database and includes survival and mortality data from radio-collared woylies, and other research components including predators, resources, and disease. The centralisation of all woylie data into the two databases has meant that tracking and interrogation of data has been made much simpler. Once all disease data has been entered from respective external collaborators it will also allow for more complex analytical diagnosis between the WCRP components.

The methodology of the database developments are discussed as well as consideration of some of the challenges associated with this exercise. These achievements are a case-model and may assist in the development toward a Corporate-wide aggregation of related datasets.

3.2.1. Introduction

Existing data relating to fauna in the Upper Warren has historically been located on multiple isolated databases, maintained by multiple custodians and in various formats. Furthermore, the currency and the extent of quality control and data validation of these databases was highly variable. Among the many shortcomings of this, was that once a problem was recognized with woylie populations rapidly declining in some places it was not possible to readily interrogate existing data to develop a comprehensive and rapid regional assessment of the extent and nature of the woylie declines.

Some datasets took months to be brought up-to-date, clearing backlogs of unentered data extending into multiple years, notwithstanding the need for data validation to improve the quality and accuracy of the data. Many of the same animal individuals were recorded in multiple independent databases, each with a unique identification and data record reference system. Similarly there were differences in the format and management of associated data including what data was collected (sex, breeding and biometrics), units of measurement (mm or cm, g or kg, etc) and quality control measures at point of data entry. As a consequence, it remained impossible to get a comprehensive record of animal individuals of interest. Until these and related issues were resolved it remained impossible to satisfactorily investigate temporal, spatial and demographic differences and changes associated with woylie decline at a regional scale.

The very large amount of DEC fauna data collected over more than 33 years within the Upper Warren, if organized into a manageable form, would serve as an extremely valuable resource that could assist substantially in the development of a reasonable understanding of the nature of the declines and provide potentially critically important evidence that could assist in the identification of the cause(s) of the woylie declines. This resource would be equally as valuable more generally, for the development of an understanding of regional fauna that would directly assist their conservation and management.

Therefore, an objective of the Woylie Conservation Research Project (WCRP) was to aggregate, as much as practically possible, the main relevant fauna-trapping datasets from the Upper Warren region into one standardised and centralised database. In addition to fulfilling the needs of the WCRP, the aim was to provide a resource that would manage all future fauna-trapping data from the region, and standardise data collection and management wherever possible and appropriate, while providing flexibility to accommodate specific requirements of independent projects. In this regard, this exercise and its product were considered a model and opportunity for the development toward a Corporate-wide tool, given that the same issues are common more broadly.

This exercise needed to be completed within a timely manner so that the aggregated data could be available for meta-analysis.

3.2.2. Methods

For efficiency, practicality and timely reorganisation of the fauna-related data, it was parsimoniously categorised into two;

- Data from fauna common to most of the principle datasets. This included trapping data and the standard biometric data of captured individuals. The principle datasets to be aggregated would include all of the 11 key transects involved in the Upper Warren Fauna Monitoring component of the WCRP. The datasets were;
  a) 'Fauna File' (1999 - 2007) – containing *Western Shield*, Fauna Management Course and other Donnelly District fauna monitoring data (Nature Conservation Division Custodians – Peter Orell, Ian Wilson/Julia Wayne)
  b) 'Kingston Project' (1994 – 2005) – containing terrestrial vertebrate data related to investigations into the impacts of timber harvesting (Science Division Custodian – Adrian Wayne)
  c) Long-term Perup fauna monitoring (1974 - 1999)– conducted by Per Christensen and Neil Burrows.
  …d) 'Bushrangers' (2000 – 2007)– a modified continuation of the long-term monitoring by Christensen and Burrows (Science Division Custodians – Bruce Ward and Graeme Liddelow)
- Data specific and unique to the WCRP, particularly the Population Comparison Study. This included data from the detailed examination of captured individuals (e.g. field health checks), samples collected from animals for associated research (e.g. disease, resources, genetics) and the results from subsequent tests on these samples, as well as data generated as part of the survival and mortality study of radio-collared woylies, predator sandpad surveys, and resources investigations.

On the basis of these classifications two distinct, but linked, databases were developed to manage these data respectively;

- Manjimup Fauna File (MFF)
- Woylie PCS

3.2.2.1. Manjimup Fauna File (MFF)

Fauna File is an existing database that was initially developed by Peter Orell in 1996 (Version 1.1 operational in 1997) for the original purpose of storing *Western Shield* data. This database grew to meet the requirements for all districts fauna trapping information and contains trapping and demographic information, as well as other fauna survey information including spotlighting and opportunistic sighting data). Fauna File has recently been revamped by Peter Orell and David McKenzie to help meet the requirements of this very large data set. Fauna File was chosen as the basis for aggregating all other data sets to create a Manjimup version of Fauna File (i.e. MFF). Donnelly District remains the custodian of the MFF.

The Woylie PCS is a database created specifically for the WCRP and contains all data for the Disease, Predator, Resource and Survivorship components of the project.

Aggregation of databases to create MFF

1. Datasets were itemised and prioritised for aggregation into MFF.
2. Datasets were located and the respective custodians enlisted for help in the aggregation process. The data was in a combination of electronic and hard copy format.
3. Standardisation of the data for importation. This involved a considerable amount of restructuring of the data into compatible fields and formatting of the data to meet the strict requirements of the database fields. Interpretation of the comments was required to match the data to the specific fields within MFF, for example, categorising reproduction comments into respective fields and matching to codes where possible. Editing measuring units. (Responsible - Peter Orell, data custodians, WCRP personnel)
4. Creation of new data fields within MFF to allow for the different information collected during various surveys. “Custom fields” were created and this allowed for the multiple fields of data within the constraints of the “Trapping Table”. (Responsible - David McKenzie and Peter Orell)
5. Creation of new fields in MFF to contain the meta-data for each of the datasets aggregated and for existing datasets. These included fields to contain information on data custodianship, background information to the custom fields, and comments on how and when the data was last updated. Provision is also made for rating each data record, one to five – nil to high level of validation, and to comment on the rating level for each data set. (Responsible - David McKenzie and Peter Orell)
6. Obtaining the original or ‘parent’ data from the respective custodians to assist with the data conversions and aggregations. (Responsible –WCRP personnel)
7. Follow a strict step by step process in MFF before trapping data can be entered. i.e. location details followed by date and personnel. Considerable effort was made to attain this information. (Responsible – WCRP personnel)
8. Importation by David McKenzie of datasets into Fauna File.
9. Data entered directly into MFF from hard copy data sets where electronic versions were not available or not suitable (Responsible – WCRP personnel and Verna Tunsell).

3.2.2.2. Woylie PCS database

Primary Objective

To create a database that would centralise all data pertinent to the WCRP.

Woylie PCS database requirements

- Fully functional during operational period of WCRP
- Efficient data entry with quality control measures at time of entry
- Security of data
- Autonomous and flexible
- User friendly
- Collate all results from the variety of external sources into the Woylie PCS database (e.g. test results from disease investigations).
- Allow for combined interrogations from different sections of the study
- Linked to Manjimup Fauna File (MFF)

Creation of Woylie PCS

1. Personnel were responsible for designing the structure and formatting of the database sections relevant to their own components of the study.
2. Information was then provided to David McKenzie for compilation and creation of the database.

3. The sections of the database were then interrogated and tested with real data. Feedback was provided to David McKenzie for any alterations and 'debugging'.
4. Over time modifications were made to the database in response to changes in data collection methods, especially with respect to disease sampling.
5. Security and quality control: The database was stored on the local network and read/write access provided to core personnel involved in the project only. Read only access was provided to other personnel.

The Woylie PCS was linked to the MFF, enabling all demographic and trapping information for an animal to be linked to the woylie PCS components for the same individuals (e.g. disease samples and field health checks).

3.2.2.3. Disease data collation

A template was provided to external collaborators for entering and returning results.

The aim being to;

1. standardise the format of the data;
2. make the process more efficient and reduce double handling of the data and;
3. most importantly to accurately match the sample results to the correct animal.

Cross-checking of samples was conducted with collaborators results and database records to account for all samples taken. This has required coordination with external collaborators. Attempts have also been made to cross-check samples that have not as yet been analysed.

Data validation of the disease data has primarily involved checking that the collaborators results are matched to the correct animal ID and that all results are captured within the database.

3.2.3. Results and Discussion

3.2.3.1. Aggregation of databases toward regional/corporate centralisation

The task involved close coordination and liaison between Peter Orell, David McKenzie, data custodians and WCRP team members to complete the aggregation process. The aggregation process was complex and difficult, requiring considerable time and effort for all those involved. The process took longer than expected due to the large number of problems with the data that were not envisaged.

The task was completed and MFF sent for meta-analysis on the 25/05/2007. The data sets aggregated into MFF are listed in Table 3.2.1. Almost 40,000 records were imported, and almost half of those were woylie records. This was a very good base for woylie meta-analysis, particularly when added to the approximately 5,000 woylie records already contained within Fauna File (PCS 990, Upper Warren 4,158).

In addition another 819 Karakamia, 49 Batalling, 32 Dryandra / Tutanning and 172 South Australian woylie records have been imported into the MFF from surveys conducted during the WCRP period.

A small percentage of records for each dataset could not be imported due to outstanding problems with the data records, in each case this was less than 5% of the data. For the Kingston dataset this was only 0.5% of the data. Data validation is still required by the custodians for the historical Boyicup and Yendicup datasets, as well as the Bushranger Yendicup and Yackelup data sets. Rating levels remain low for these data sets at present.

Importation issues

Ear tag clashes were the greatest problem in the importation process. The overlap in sites and survey periods between the different datasets caused problems determining whether ear tags were from the same or different animals. The occurrence of duplicated ear tag numbers was also a significant problem, particularly ear tags with no prefix. A central or at least co-ordinated control of the ear tags codes used by different operators is highly recommended to avoid this problem continuing in the future.

The dataset that was by far the most difficult to import was the Kingston dataset that was stored within a similar, but not identical database to Fauna File. The small differences created a number of issues for importation. For example, right and left ear tag order was not necessary in the

Kingston database. The animal was recognized either way. In Fauna File, however this was important in animal recognition.

There were also the inherent database issues of linked tables. The main table that holds the data is linked to several other background tables. If the person initially entering the data is not aware of this they can create erroneous records within these linked tables. These erroneous or "ghost" entries had to be fixed prior to importation, slowing down the process immensely.

The requirement to collect the original or 'parent' hardcopy data prior to importation of the actual trap data and restructuring of the data to meet the specific field criteria also slowed down the aggregation process.

Table 3.2.1. Datasets integrated with the original Fauna File datasets to create the 'Manjimup Fauna File' (MFF).

Data Source	Survey period	Custodian	Total No. records imported	No. woylie records
Kingston	1994-2005	Adrian Wayne	29151	>12000
Boyicup	1974-1986	Neil Burrows / Per Christensen	4088	3142
Yendicup	1977-1998	Neil Burrows / Per Christensen	1666	1397
Bushranger (Yendicup)	2000-2007	Bruce Ward / Graeme Liddelow	2165	940
Bushranger (Yackelup)	2000-2007	Bruce Ward / Graeme Liddelow	2055	590
Total			**39125**	**>18069**

NB: 1999 Boyicup data not aggregated into the MFF due to limited baseline information.

3.2.3.2. Creation of Woylie PCS database

Sections of the Woylie PCS database were operational in late 2006 and sections were added as designed by WCRP personnel. David McKenzie had completed all sections, including any required alterations by June 2007. All database sections were operational and running smoothly in mid 2007, in time for the bulk of the data entry to be completed and analyses to begin shortly thereafter. The development of Woylie PCS database based on current data requirements was comparatively easier and much quicker than the aggregation of data into MFF.

All components of the study, other than "demographics" were included within the Woylie PCS database (Figure 3.2.1). Table links with MFF enabled full capacity for the relational data to be integrated and interrogated accordingly. In eventuality the Woylie PCS database contains data from Perup (PCS and Upper Warren sites), Karakamia, Batalling, Dryandra, Tutanning and South Australia.

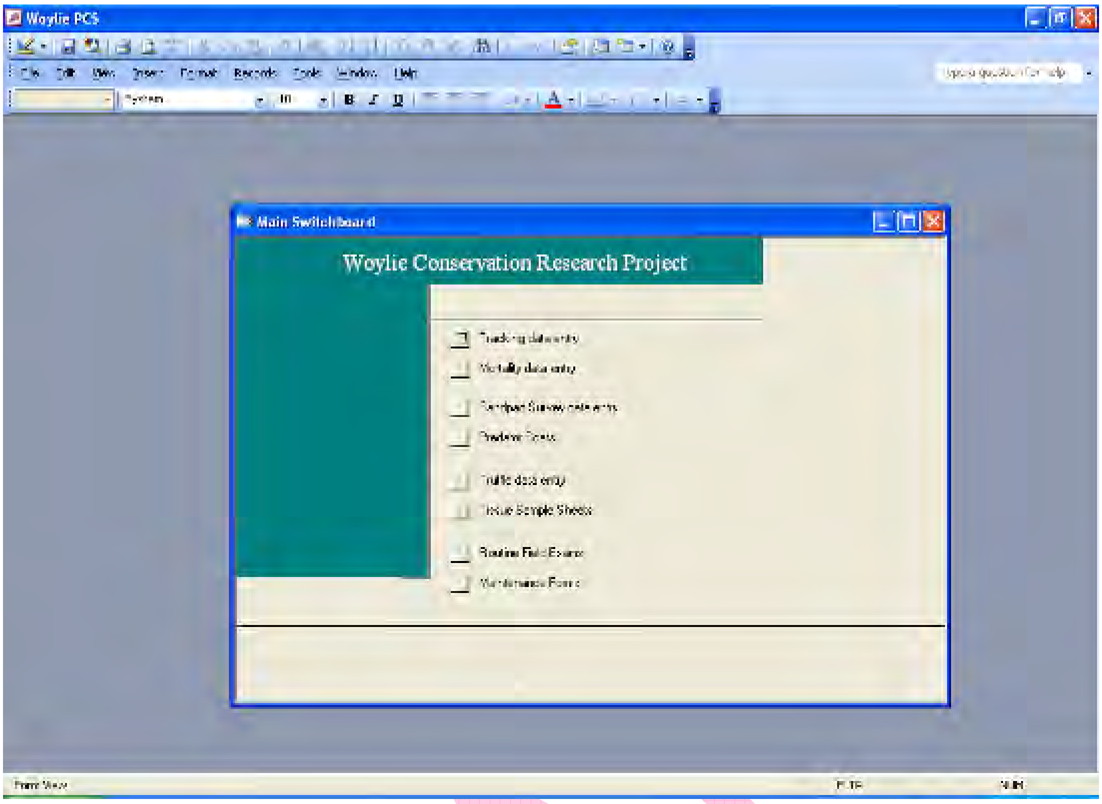

Figure 3.2.1. Woylie PCS database main menu.

3.2.3.3. Disease data collation

The results received from external collaborators and consultants have been entered into the Woylie PCS database by WCRP personnel and DEC casual employees. Therefore, for every animal that has had any sample taken there is a complete history of disease and demographic information.

The data has been cross-checked and validated with the custodians of the results as much as possible. Some work is still required before these datasets can be comprehensively interrogated with associated relational data.

Importation issues

The format of results from external collaborators and consultants were not consistently standardised as requested. It was particularly difficult to match samples to animals due to collaborators using their own reference numbers. The independent data was also returned in a variety of formats (i.e. Word documents, Excel spreadsheets, etc) and the structure varied as did the content. As a result, in the majority of cases the data had to be entered manually into the database rather than imported as intended. There are still current validation issues with outstanding records that have not as yet been positively matched to the animals from which they came. There will be an ongoing requirement for importation of disease results as they are made available from the external collaborators and consultants.

3.2.3.4. Data management and intellectual property

The intellectual property rights associated with the datasets provided by multiple custodians has been a high priority for the management of these datasets. This is especially important given the high level of involvement of individuals from a number of institutions and agencies. While fundamentally based on trust, co-operation and a conducive culture, the use of the data is managed through a data request process that registers the use, its purpose and gets approval from the original authors/custodians of the data (Volume 3 Data-use conventions and Data request form).

3.2.4. Future work

- To achieve the goal of centralisation of fauna data sets it is very important that unique ear tag prefix and numbers are used when conducting fauna surveys.
- Reinforcing and developing the standardisation of data collection methods and units of measure for fauna surveys.
- Database training or close supervision for personnel entering data to prevent the creation of “ghost” records within the database.
- Some improvements to the databases could be made to enhance the quality control provisions at the time of data entry and to facilitate data validation efforts.
- Ongoing maintenance of the MFF to both update and validate existing and historical data in conjunction with the custodians of the aggregated data sets.
- Web based Woylie PCS database that would allow external collaborators and consultants to enter their results directly into the database and enable them to cross check and validate their own data in relation to the individuals from which the samples were originally collected.

3.2.5. Conclusion

- Successful aggregation into the Manjimup Fauna File (MFF) of multiple, previously independent datasets associated with the Kingston Project, long-term monitoring of Perup, Bushrangers, *Western Shield*, DEC Fauna Management Course, and Donnelly District fauna activities.
- The Woylie PCS database was created to centralise all data pertinent to the WCRP including survival and mortality data from radio-collared woylies, predator, resource, and disease components of the study. This database was linked to the demographic data within the MFF to provide an easily obtainable complete history of disease and demographic information for each individual animal.

Practical advantages of the aggregation include;

- Comprehensive relational history of individual animals involved in multiple fauna programs.
- Substantial progression towards standardisation of data collection and management, including improved data quality control.
- Optimises the value of the data collected over more than 30 years.
- Facilitated efforts to diagnose the woylie declines in the Upper Warren, particularly by providing the data available for the subsequent meta-analyses.
- Provision of a useful model and case study for considerations of the development of a corporate-wide aggregation of similar datasets.

3.2.6. Acknowledgements

We would like to thank Sheryn Prior for her short but invaluable data management role and in particular her persistence and diligence with the preparation for importation of the historical Boyicup and Yendicup datasets. We would also like to thank Lauren Daubney and Verna Tunsell for their data entry contribution.

Marnie Swinburn, in her roles as Deputy Disease Coordinator and External Programs Liaison, was responsible for liaison with external collaborators and ensuring provision of data for both the Woylie PCS as well as to the requesting collaborators.

3.3. Population change – temporal, demographics, other species

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Abstract

Cage trapping data on woylies collected through a number of studies between 1994 and 2007 at fifteen sites in the Upper Warren region was analysed to better understand population trends and in particular to test the hypothesis that the population is currently in decline. To the extent that this analysis used data from independent studies, it was a meta-analysis and needed to cope with significant variation in the quality and detail of the data. However the raw nightly trap data was used in all cases and the analysis used both linear regression and a generalised linear model approach with a "quasibinomial" error term to allow for the variation between studies. The trapping data provides statistically strong evidence that woylie numbers have been in decline since 2002. The data is less clear on what might be the cause of this decline. A significant positive correlation was found with trapping of quenda, but no strong association was found with koomal or chuditch. Inconsistent trends were observed in the proportion of males captured at each site and the condition indices showed no significant pattern with regards to time or site. Since almost all the woylies caught were adults (95%), little could be said about trends in population structure. Similarly, no significant patterns were found in their weights or size except for the expected sex and age differences.

3.3.1. Introduction

Data Analysis Australia was contracted by the Department of Environment and Conservation to provide a meta-analysis of live cage-trapping data they collected within the Upper Warren region (east of Manjimup).

The woylie (*Bettongia penicillata*) was removed from the endangered list after the *Western Shield* fox baiting program and translocations lead to a recovery in woylie numbers. Recent investigations have suggested that there may be a substantial decline in the abundance of woylies in the southwest forests of Western Australia. Although this decline has not yet been statistically verified, indications suggest the decline to be extreme enough that immediate action needs to be taken, including the possible reinstalment of woylies to the endangered species list.

Trapping data within the Upper Warren region includes long-term monitoring research (P. Christensen and N. Burrows), the 'Kingston Study' into timber harvesting impacts and associated research (A. Wayne), *Western Shield* monitoring transects and other Donnelly District activities (I. Wilson).

The work undertaken by Data Analysis Australia has two aims:

1. A definitive and independent review of the historical trapping data to give an objective statement regarding the decline (or otherwise) of the woylie population; and
2. An exploration of the nature of the decline is needed, assuming that statistical evidence of a decline is found in the first stage. This exploration includes an analysis of the population demographics as well as other measures collected during the monitoring program.

3.3.2. Data

Monitoring programs have been set up as part of the Western Shield program and DEC Science Division research projects to monitor for changes in native fauna. Road transects were commonly chosen on the basis of practical considerations such as accessibility. Usually these transects are repeated but are not always consistent and are generally carried out over 4 consecutive nights. The majority of the data was collected during the autumn months.

The information available for analysis consisted of trapping data relating to the number and characteristics of woylies and other species collected from selected sites during discrete time intervals from March 1994 until April 2007. Data was only included for 'CS' (cage small) traps where universal bait was used.

Table 3.3.1 lists the survey units that constituted each site, together with the time period over which data was available for that particular site. Table 3.3.2 lists the trapping quantities that were used in the analyses, namely the total number of trapping sessions that occurred for each site and the total number of traps that were set for each site. For the purposes of these analyses, a single trapping session for the site in question refers to the total number of trapping nights that occurred in a particular month and year. For example, Balban's three trapping sessions occurred on November 2004, March 2006 and March 2007. Table 3.3.3 and Table 3.3.4 illustrate the gender and age distribution of the woylies that were captured at each site.

The data collected at each site was done by different personnel, for different programs, thus different levels of detail was recorded for each individual woylie. For example, the age and sex of individuals subsequently retrapped (i.e. repeated captures) within the same trap session are not routinely recorded by some operators. This largely explains the differences in the proportion of captured woylies with either unknown (not recorded) gender or unknown (not recorded) age. While this does not necessarily invalidate the analysis that follows, it does suggest a degree of caution is required when comparing sites.

Earlier data from the Boyicup and Yendicup sites that dated back to 1974 could not be used as the total number of sample points corresponding to each trapping session was not recorded in the database. This made it impossible to determine the number of traps available or calculate capture rates for these two data sets.

Table 3.3.1. Summary of data available for analyses.

Site	Survey Unit	Data Available
Balban	51FMC/02	Nov 2004 - Mar 2007
Boyicup	51BOY/01	Mar 1998 - Mar 2007
Camelar	51FMC/01	Nov 2000 - Mar 2007
Chariup	51CHP/01	Mar 1998 - Mar 2007
Corbal	51COR/01	Dec 2005 - Apr 2007
Keninup1	51WSK-01	Jan 1999 - Jul 2002
Keninup2	51KEN/01	Nov 2005 - Mar 2007
Kingston	C1-C4, KC-5, KC-6	Mar 1994 - May 2005
Moopinup	51POS/01	Mar 1996 - Mar 2007
Warrup1	ED, K5NB, K5WB, NFK, SR1-SR3, WL, WRP	Mar 1994 - Aug 1998
Warrup2	51WAR/01	Feb 2001 - Apr 2007
Winnejup1	KNW, KN1, KN2, LJN, TWR	Apr 1994 - Aug 1998
Winnejup2	WEB, WNB	Apr 1994 - Apr 2007
Yackelup	51YAU/01	Oct 2000 - Mar 2007
Yendicup	51YEU/01	Mar 2000 - Mar 2007

Prior to analysis, data was aggregated on a monthly basis for each site. Thus time was recorded in terms of month and year. For ease of analysis, this was converted to a continuous variable by assigning the number 1 to the month of January 1994, number 2 to the month of February 1994 and so on until the number 160 was assigned to the month of April 2007.

Table 3.3.2. Number of trapping sessions and number of traps per site that were used in the analyses.

Site	Trapping Sessions	Number of Traps
Balban	3	600
Boyicup	12	2,400
Camelar	9	1,800
Chariup	11	2,300
Corbal	4	800
Keninup1	7	175
Keninup2	4	800
Kingston	36	6,480
Moopinup	14	2,684
Warrup1	22	7,038
Warrup2	9	1,800
Winnejup1	21	3,446
Winnejup2	24	4,818
Yackelup	14	1,050
Yendicup	15	1,125

Table 3.3.3. Gender distribution of captured woylies.

Site	Captured Woylies		
	Males	**Females**	**Unknown**
Balban	158	91	1
Boyicup	458	308	2
Camelar	191	77	16
Chariup	512	280	9
Corbal	72	38	2
Keninup1	40	20	1
Keninup2	247	163	11
Kingston	807	587	29
Moopinup	483	304	4
Warrup1	1,092	724	209
Warrup2	239	149	7
Winnejup1	788	592	132
Winnejup2	495	355	132
Yackelup	252	116	20
Yendicup	317	214	26

Table 3.3.4. Age distribution of captured woylies including new and recaptured individuals, and retrap events of the same individual within the same session.

Site	Captured Woylies				
	Adult	Subadult	Juvenile	Infant	Unknown
Balban	232	5	1	1	11
Boyicup	750	5	9		4
Camelar	265	5	10	1	3
Chariup	753	12	16		20
Corbal	94	1	1	2	14
Keninup1	60		1		
Keninup2	350	14	9	12	36
Kingston	1,318	8	35	9	53
Moopinup	764	21	3		3
Warrup1	1,051	23	117	3	831
Warrup2	365	7	7	6	10
Winnejup1	732	17	56	6	701
Winnejup2	467	10	30	3	472
Yackelup	340	1	17		30
Yendicup	505	1	16	1	34

3.3.2.1. Issues

Data Analysis Australia noted several key issues from this work. The lack of data available for each site would restrict the accuracy of the model in terms of the temporal and demographic characteristics of woylies. Trapping was also not performed consistently, with temporal gaps where traps had not been laid at some sites for a number of years. In addition, sites such as Winnejup and Warrup underwent changes such that the number of traps laid in later years was only a subset of those that had been laid in previous years. Such gaps in the data limit the ability of any model to estimate when a decline in the woylie population may have occurred.

The data available was not collected specifically for the purpose of this analysis, explaining some of the inconsistencies found in the dataset. Trapping data was entered by various individuals, increasing the potential for errors resulting from data entry and the varying perceptions that different individuals had when determining such demographics as the age of a woylie. The Department of Environment and Conservation has been processing and validating the data to overcome such issues although this process had not been completed at the time of the analysis.

A particular issue related to the classification of adult female woylies as breeding or not breeding. This breeding status was not recorded consistently at individual sites and consideration of a number of data fields was required to perform this calculation.

Breeding was assumed to be occurring if, for a particular woylie, at least one of the following was recorded in the data:

- Pouch activity described as active, lactating or suckling;
- Number of pouch young ≥ 1;
- Crown rump length of pouch young provided;
- Number of elongated teats ≥ 1; or
- Comments field made reference to elongated/enlarged teats, pregnancy or condition of the young.

In the raw data, zeros in the relevant data fields were sometimes used to indicate that a woylie was not breeding whilst, on other occasions, data fields were simply left blank. It is unknown whether data fields were kept blank as a result of the woylie's breeding status not being recorded or because the woylie was not currently breeding. In both instances, a woylie was assumed to not be breeding. For the purposes of analysis, the assumption had to be made that the lack of recording of breeding status was consistent across the various sites.

This resulted in the creation of a new variable that assigned a value of 1 to those adult female woylies that were breeding and 0 to those that were not.

In saying this, the data that has been collected is extensive and is the richest source of woylie data available for the analysis. Despite its limitations, the data was sufficient to undertake substantial analysis and draw meaningful results – the results simply need to be interpreted with the data limitations in mind.

3.3.2.2. Statistical techniques

A multistage conditional approach was adopted for the analysis, examining in sequence the population size, then the demographics (age and gender) and finally condition (weight and size). By making each analysis conditional on the previous stages relationships could be examined using generalised linear models, an extension to standard regression.

Linear regression attempts to model a numeric response variable, *y*, by a linear combination of predictor variables x_j, for *j*=1…p. Each of these variables was observed for the same *n* observations. The fitted values are the sum of the coefficients β_j multiplying each of the x_j plus an intercept β_0. In other words:

$$y \sim \beta_0 + \beta_1 x_1 + ... + \beta_p x_p$$

Using the standard statistical theory the model for the *i*th observation can be written as:

$$y_i = \beta_0 + \sum_{j=1}^{p} \beta_j x_{ij} + \varepsilon_i$$

and by making the following assumptions:

1. the ε_i are independently and identically distributed;
2. the ε_i have mean zero and (finite) variance σ^2; and
3. the ε_i are distributed according to the normal distribution.

This model was used to evaluate the numeric response variables: weight, hindfoot length and head length as part of the woylie population health analysis.

However, the data also contained many factor variables. Factor variables are those in which one coefficient is given for each level. The coding of factors into the model involves replacing the factor by one "dummy" variable for each level – namely, a numeric variable taking the value 1 wherever the factor takes on that level, and 0 for all other observations. For example, this analysis involved the factor variable Sex, in which *XMale* is set to 1 for all Male observations and *XFemale* is set to 1 for all Female observations. Therefore, the model becomes:

$$y \sim \beta_0 + \beta_1 XMale + \beta_2 XFemale\,.$$

Occasionally, the parameterisation of a term may need to be handled explicitly. For a factor with *k* levels, *k*-1 such linear combinations are possible. A particular choice of these linear combinations is called a contrast, or *reference group*. Any choice of reference group for factors alters the specific individual coefficients in the model but does not change the overall contribution of the term to the fit. In terms of the analysis completed on the trapping data, the site Kingston was chosen as the reference group for all the models as it contained the most data points when compared to the other sites that were monitored and had a low proportion of trapped woylies for which the gender or age were not recorded.

Due to the discrete response variables involved a generalised linear model was employed to look at changes in the woylie population and demographics. This was a natural progression from using linear regression models. Generalised linear models deal with issues such as binary response data (i.e. where 1 = success and 0 = failure) by using re-parameterisation to induce linearity and by allowing a non-constant variance to be directly incorporated into the analysis. Many of the variables used in the analysis can be interpreted as being one of two outcomes, i.e. success or failure, or as a binary response.

When dealing with binary data, the logit link function, $log\{\mu/(1-\mu)\}$, is employed which is used to describe how the mean depends on linear predictors. This link guarantees that μ is in the interval (0,1), which is appropriate since μ is a proportion.

The introduction of a quasibinomial model allows for the distribution to be specified entirely by the mean and variance functions for binary variables, whilst allowing for additional variation in the data compared to that which would be expected under the binomial distribution. This additional variation is expected for variables such as the trapping rates since it is expected that each trap night at a site is statistically dependent of other trap nights. The deviation above that of the binomial distribution is given by the dispersion parameter with a value of 1 equalling the binomial distribution and larger values showing a larger variation. In tests for the significance of relationships the quasibinomial model correctly accounts for the additional variation whereas the more common binomial model tends to over state the significance.

3.3.2.3. Condition index

The (body) condition index attempts to quantify various attributes, which describe the general health and fitness of individuals within a population. This index relates body mass (weight) to sex, length (both hindfoot and head length were assessed) and the interaction between these two attributes.

For the adult woylie individuals involved in this study, there were multiple measures of the same individual over time. In this model, within- and between- woylie variation was accounted for by using a linear mixed model in which woylie identity (ID) was included as a random effect. Fixed effects included the sex, hindfoot and head length of the individual and the interaction between the length parameter and sex.

Both the fixed effects model and the fixed effects model with a random effect were assessed and it was found that the model that incorporated the random effect was a better model based on the goodness-of-fit.

The sites chosen for this analysis were based on their underlying quadratic structure, as the condition index is best represented with reference to time (discussed further in Section 3.3.3).

Two models were formulated based on the type of length measurement used. It was found that head length was a better measurement than hindfoot length in characterising the condition index of individual woylies. Therefore the final model used in calculating the condition index involved the fixed effects sex, head length and their interaction, and the random effect ID. The residuals of this model provided a condition index that was specific for each individual and relative to its own ‘usual’ condition. This model showed a significant relationship between head length ($p<0.001$), sex ($p<0.01$) and their interaction ($p<0.01$).

Exploratory plots were conducted on the condition index to assess what the most appropriate parameter would be to use in the population decline model.

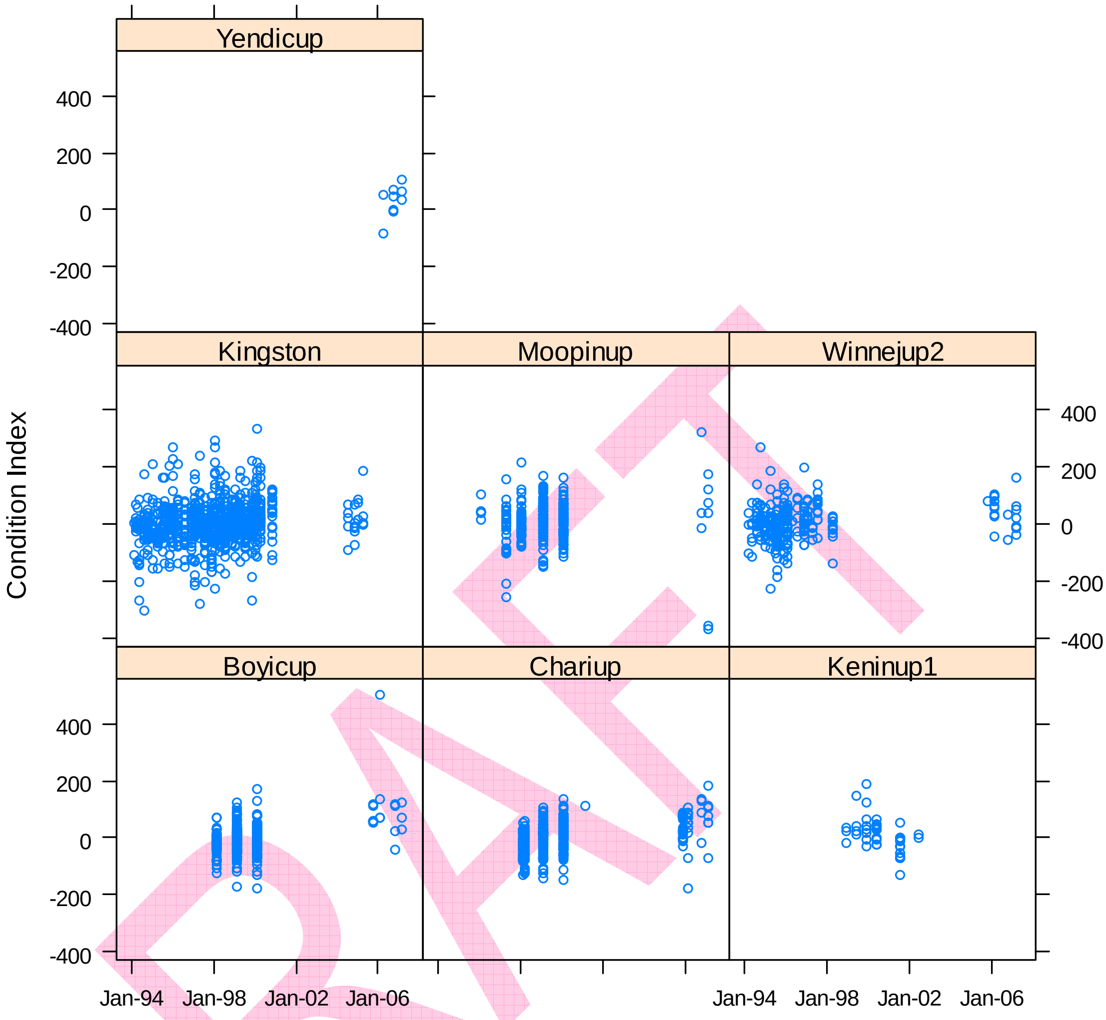

Figure 3.3.1. The condition index for individual adult woylies over time for each site.

The condition index showed that the majority of the adult woylie individuals were between ±200 grams of their 'usual' condition. Figure 3.3.1 also shows that there is no obvious inherent pattern in the dataset.

To further ascertain if there are any trends in the data, the average and standard deviation of the condition index was evaluated for each site at each time where data was available.

Inspection of the average condition index (Figure 3.3.2) shows that there appears to be an increasing trend in some sites over time. This suggests that there may be an increase in the overall health of the population at the sites Boyicup, Chariup, Kingston and Yendicup, whereas the sites (Moopinup and Winnejup2) are more static. Keninup1 is the only site to have experienced a possible decrease in their condition index over a short amount of time.

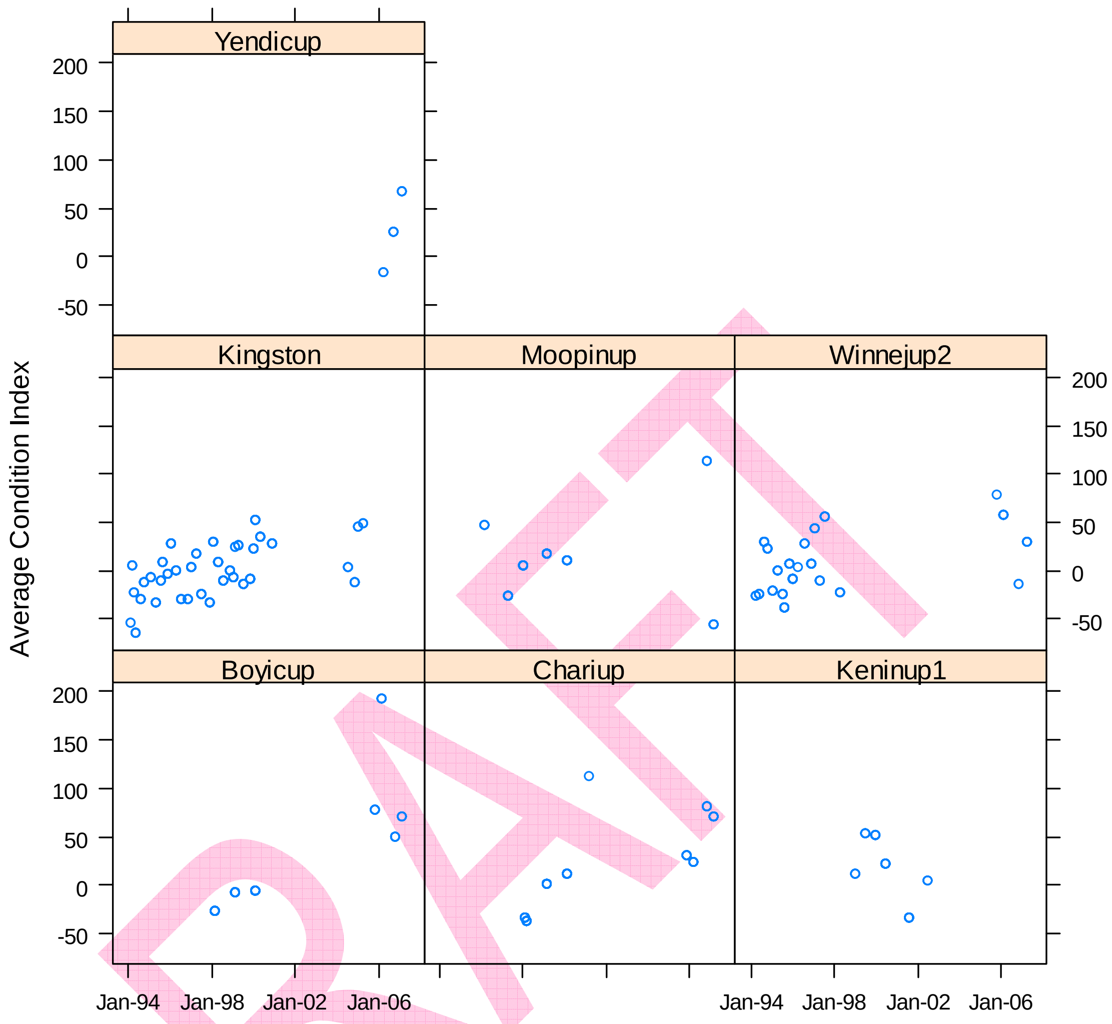

Figure 3.3.2. The average condition index over time for each site.

The standard deviation of the condition index was also examined and it was found that there was no apparent change over time for the sites Boyicup, Chariup, Keninup1, Kingston, Moopinup, Winnejup2 and Yendicup.

3.3.3. Population decline

A generalised linear model was developed in an attempt to characterise the trapping of woylies, investigate whether there has been a population decline and determine the significant factors that may have contributed to this decline. Essentially the probability of catching a woylie was modelled, based on time and other potential explanatory variables. While this is not a direct measure of population size, under all standard models for trapping rates it is closely associated with population size and is expected to have more understandable statistical properties than other measures.

The trapping of a woylie was modelled in terms of successes and failures. A success was defined as the capture of a woylie, irrespective of whether it was new, recaptured, escaped, not tagged or had not been identified under any of these categories. A failure was defined as not capturing a woylie and could therefore be either an empty trap or a trap that had captured a species other than a woylie.

The model was only applied to data from a selected number of sites.
Table 3.3.5 shows the sites that were included and those that were excluded from the model.

Table 3.3.5. Sites on which the model of population decline was based.

Sites Included	Sites Excluded
Boyicup	Balban
Chariup	Camelar
Keninup1	Corbal
Kingston	Keninup2
Moopinup	Warrup1
Winnejup2	Warrup2
Yackelup	Winnejup1
Yendicup	

Sites were excluded when the period of data collection showed that the population of woylies, denoted by the probability of successfully capturing a woylie, was either monotonically increasing (i.e. strictly increasing or non-decreasing) or monotonically decreasing (i.e. strictly decreasing or non-increasing) rather than covering the likely timing of when the population changed (based on that observed at other sites). These monotonic patterns are shown in Figure 3.3.3 where the probability of success of capturing a woylie was plotted for each of these excluded sites over time.

The probability of success of capturing a woylie was then plotted over time for each of the remaining sites that were included in the model and displayed in Figure 3.3.4. The pattern in the data in Figure 3.3.4 suggests there is a period of increase in the numbers of woylies caught, followed by a decrease in the number of woylies caught. This is in stark contrast to the monotonic nature of the data from the excluded sites shown in Figure 3.3.3.

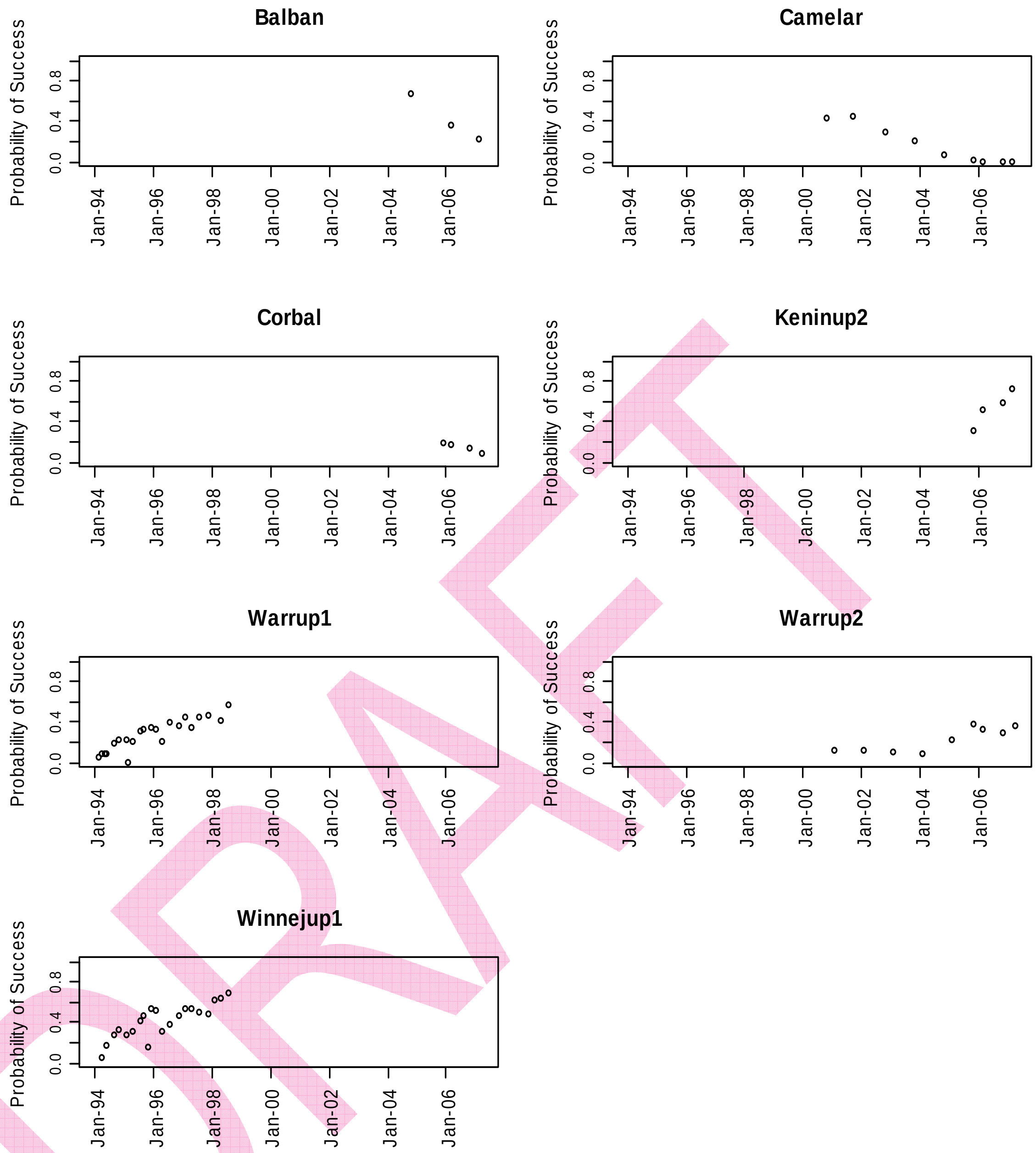

Figure 3.3.3. Probability of success (catching a woylie) over time for sites Balban, Camelar, Corbal, Keninup2, Warrup1, Warrup2 and Winnejup1.

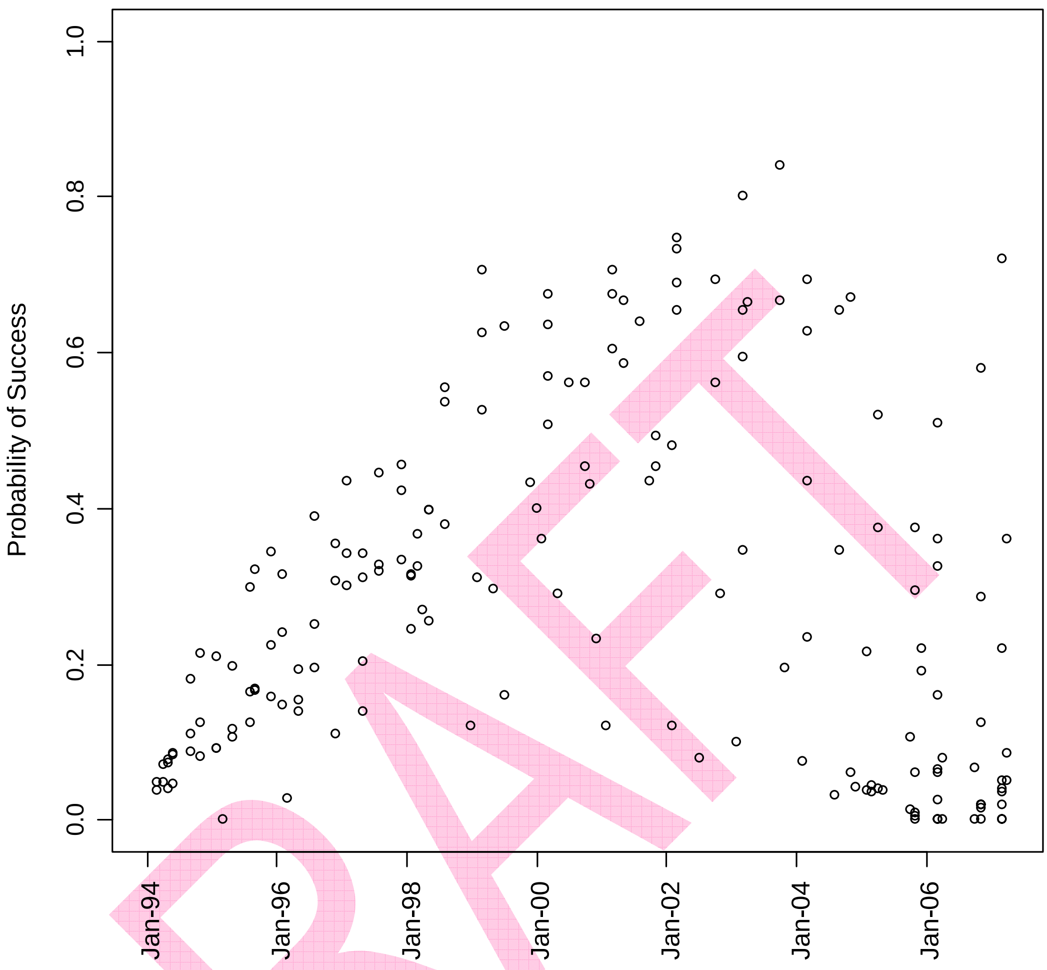

Figure 3.3.4. Probability of capturing a woylie over time for selected sites that were included in the model of population decline of woylies.

Data was only available for three or four trapping sessions for sites such as Balban, Corbal and Keninup2. As the plots for these sites clearly show, there is insufficient data available to indicate when the probability of capturing a woylie shifted from an increase to a decrease. For sites such as Warrup1 and Winnejup1 it was not possible to accurately estimate the time of the decline in numbers of woylies, since the probability of catching a woylie followed an increasing trend and no data was available after 1998. The absence of data prior to 2001 for Camelar and Warrup2 also made it impossible to accurately estimate a decline in the woylie population. For these reasons, these sites were excluded from the model.

The resulting model of population decline, based on the selected sites, is shown in Table 3.3.6.

The statistically significant variables that were used in the model to explain the capture of a woylie consisted of Time, $Time^2$, $Time^3$, Site and the interaction between time and site (Time*Site).

Table 3.3.6. Quasibinomial model where successful capture of a Woylie = Time+Time^2+Time^3+Site+Time*Site and failure is an empty trap or a species captured other than a woylie. ('*' Pr<0.001, '**' Pr<0.01, '*' Pr<0.05)**

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	-2.545	0.277	-9.21	1.84E-15	***
Time	0.026	0.014	1.85	0.07	.
Time_squared	3.96E-04	2.14E-04	1.85	0.07	.
Time_cubed	-4.96E-06	9.11E-07	-5.45	2.96E-07	***
SiteBoyicup	0.128	0.5673	0.23	0.82	
SiteChariup	-2.058	0.4759	-4.33	3.27E-05	***
SiteKeninup1	-2.335	2.3680	-0.99	0.33	
SiteMoopinup	-1.481	0.4550	-3.26	0.00	**
SiteWinnejup2	-0.414	0.2714	-1.53	0.13	
SiteYackelup	-2.097	1.2460	-1.68	0.10	.
SiteYendicup	-3.748	0.9846	-3.81	0.00	***
Time:SiteBoyicup	0.012	7.06E-03	1.75	0.08	.
Time:SiteChariup	0.041	6.31E-03	6.50	2.14E-09	***
Time:SiteKeninup1	0.029	0.028	1.03	0.30	
Time:SiteMoopinup	0.029	6.05E-03	4.82	0.00	***
Time:SiteWinnejup2	0.020	5.59E-03	3.65	0.00	***
Time:SiteYackelup	0.036	0.012	2.98	0.00	**
Time:SiteYendicup	0.056	9.70E-03	5.80	6.07E-08	***

Dispersion parameter for quasibinomial family taken to be 6.447442

Residual deviance: 637.18 on 115 degrees of freedom

The terms that were significant in explaining the capture of a woylie all had a p-value of 0.004 or less and included the following:

- Time^3,
- Sites of Chariup, Moopinup and Yendicup;
- Interaction of Time with the sites of Chariup, Moopinup Winnejup2, Yackelup and Yendicup.

Other explanatory variables were investigated for the model such as cosine[1] and sine terms to model any possible seasonal variation in the data, and the average condition index. However, these were found to be not significant.

The proportion of adult woylie females that had been recorded as breeding (represented as a proportion of the total number of adult female woylies captured) was also investigated as an explanatory variable for the model yet it was not found to be significant.

It was not appropriate to include explanatory variables in the model such as the numbers of various other species captured since these other species are included in the definition of a failure. Instead an alternative model was developed that was restricted to the same sites as the model described in Table 3.3.6 yet enabled the significance of other captured species to be tested. The capture of a woylie was modelled in terms of successes and failures. However, whilst the definition of a success remained the same, a failure was now defined as an empty trap. In other words, the model was only taking into account the available traps that remained after another

[1] The cosine and sine terms were calculated according to the equations:

cosine term=cos((2*pi* Time)/12),

sine term=sin((2*pi* Time)/12),

Such terms give the model a seasonal component. This definition of the cosine and sine terms was used throughout this paper for all models investigated.

species had been captured. This was in contrast to the model shown in Table 3.3.6 that considered all traps that had been set. The resulting model in which the capture of a woylie is explained by the statistically significant variables Time, $Time^3$, Site and the Time*Site interaction term, is shown in Table 3.3.7.

Table 3.3.7. Quasibinomial model where successful capture of a woylie = Time + $Time^2$ + $Time^3$ + Trap Rate for koomal + Trap Rate for quenda + Site + Time*Site and failure is an empty trap. ('*' Pr<0.001, '**' Pr<0.01, '*' Pr<0.05)**

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	-3.072	0.445	-6.90	3.22E-10	***
Time	0.041	0.018	2.32	0.02	*
Time_squared	3.93E-04	2.60E-04	1.51	0.13	
Time_cubed	-5.40E-06	1.13E-06	-4.79	5.06E-06	***
TrapRate_BTP	1.639	0.841	1.95	0.05	*
TrapRate_Quenda	4.232	1.450	2.92	0.00	**
SiteBoyicup	0.069	0.707	0.10	0.92	
SiteChariup	-2.234	0.596	-3.75	0.00	***
SiteKeninup1	-2.199	2.472	-0.89	0.38	
SiteMoopinup	-1.464	0.560	-2.61	0.01	*
SiteWinnejup2	-0.736	0.356	-2.07	0.04	*
SiteYackelup	-2.422	1.376	-1.76	0.08	.
SiteYendicup	-4.277	1.059	-4.04	9.79E-05	***
Time:SiteBoyicup	9.07E-03	7.60E-03	1.19	0.24	
Time:SiteChariup	0.040	6.66E-03	6.02	2.24E-08	***
Time:SiteKeninup1	0.022	0.029	0.76	0.45	
Time:SiteMoopinup	0.025	6.53E-03	3.85	0.00	***
Time:SiteWinnejup2	0.021	6.11E-03	3.51	0.00	***
Time:SiteYackelup	0.034	0.013	2.72	0.01	**
Time:SiteYendicup	0.057	0.010	5.62	1.41E-07	***

Dispersion parameter for quasibinomial family taken to be 6.736329

Residual deviance: 614.27 on 113 degrees of freedom

The models shown in Table 3.3.6 and Table 3.3.7 were very similar with all variables that were significant in the first model also being significant in the second model. In addition Time, Winnejup2, the Trap Rate for common brushtail possums (defined as the number of common brushtail possums caught divided by the number of traps available) and the Trap Rate for quenda (defined as the number of quenda caught divided by the number of traps available) were also significant in the second model.

Other explanatory variables such as cosine and sine terms, the average condition index and the proportion of adult woylie females that were recorded as breeding were again investigated for the model yet these were not found to be statistically significant. Whilst the trap rate for quenda and common brushtail possum was significant in explaining the capture of woylies, thus indicating that there is some level of association with woylies, the trap rate for chuditch was found to be not significant.

Since both of the models described in Table 3.3.6 and Table 3.3.7 are quasibinomial, it is difficult to determine, in absolute terms, how well the models fit the data. If it was not necessary to allow for additional variation in the data and hence a binomial model could be applied then the goodness-of-fit could be ascertained according to whether the variance in the data was higher or lower than the expected variance from the binomial distribution. Whilst a quasibinomial model assumes that the variance in the data will deviate from the variance under the binomial distribution, the actual value of this expected variance is unknown. Thus an absolute measure of goodness-of-fit cannot be determined.

In addition, the absence of any genuine replication within the trapping data results in the data being too sparse and this consequently renders the p-values generated by any goodness-of-fit tests to be invalid. Whilst the residual deviance may provide a relative measure of the goodness-of-fit, when compared to the residual deviance produced by other models that were applied to the dataset, a large residual deviance could be explained by a poor fitting model or by the variation in the data being naturally greater than that assumed by the model.

Based on the model, Table 3.3.8 shows the approximate time at which the population of woylies began to decline at each site. The table lists sites in order of the earliest site to show a decline that was estimated by the model, through to the most recent site.

Table 3.3.8. Estimated commencement dates of decline according to model using selected sites versus visually estimated date of decline.

Site	Modelled Date of Peaking	Visually Estimated Date of Decline
Kingston	August 2000	1999
Boyicup	January 2001	2002 to 2003
Keninup1	July 2001	2001 to 2002
Winnejup2	July 2001	1998 to 2005
Moopinup	September 2001	2001 to 2002
Yackelup	January 2002	2004
Chariup	March 2002	2002 to 2004
Yendicup	October 2002	2004
Balban	NA	Pre 2006
Corbal	NA	Pre 2006
Camelar	NA	2002 or earlier
Keninup2	NA	Increasing trend in data
Warrup1	NA	Increasing trend in data
Warrup2	NA	Increasing trend in data
Winnejup1	NA	Increasing trend in data

Separate plots of the probability of success of capturing a woylie over time were produced for each site that was included in the model. These plots are shown in Figure 3.3.5. The estimated time of decline in the number of woylies for each site is shown by the vertical line and is only approximate as issues with the data made it difficult to estimate the time of decline in the number of woylies. There was only limited data available and inconsistency in trapping resulted in missing data for some sites due to traps not being set in all years. For example, a decline in the woylie population at Winnejup2 could have occurred any time between December 1998 and November 2005 since no traps were laid at that site during that time period.

There was also difficulty in estimating the time of decline in numbers of woylies for Yackelup and Yendicup due to the erratic nature of the data. Figure 3.3.5 shows that, whilst the probability of capturing a woylie appears to decline at Yackelup after approximately December 2003, the alternating increasing and decreasing probabilities prior to this date result in an estimated date of decline in March 2001. Figure 3.3.5 also exhibits a similar scenario for Yendicup.

For the models in Table 3.3.6 and Table 3.3.7, sites 1 and 2 for Keninup, Warrup and Winnejup were kept separate. This separation represented changes in the sampling methodology where trapping methods had changed such that site 2 was only a subset of the number of traps that were laid for site 1. Sites 1 and 2 were combined for Keninup, Warrup and Winnejup and a model was applied to the resulting dataset, using the same definition of failure as the model in Table 3.3.7. Table 3.3.However, as Table 3.3.9 shows, this resulted in less accurate and realistic estimated times for the decline in the population of woylies at the various sites when these were compared to the rough estimates for the time of decline that were obtained by visually inspecting the plot of successes over time for that site that are shown in Figure 3.3.3 or Figure 3.3.5.

When combining sites we must be reasonably sure that the changes between sites 1 and 2 are small or they can somehow be incorporated into the model. Changes in sampling methodology may or may not cause substantial changes in the data. The less accurate model estimates for the time of the population decline that were produced after combining the sites (shown in Table 3.3.9), compared to those times estimated by the model where the sites remained separate (shown in Table 3.3.8), suggest that there are differences in the data from sites 1 and 2 that extend over and above the changes in sampling methodology. For this reason, it is recommended that sites 1 and 2 should be kept separate for Keninup, Warrup and Winnejup.

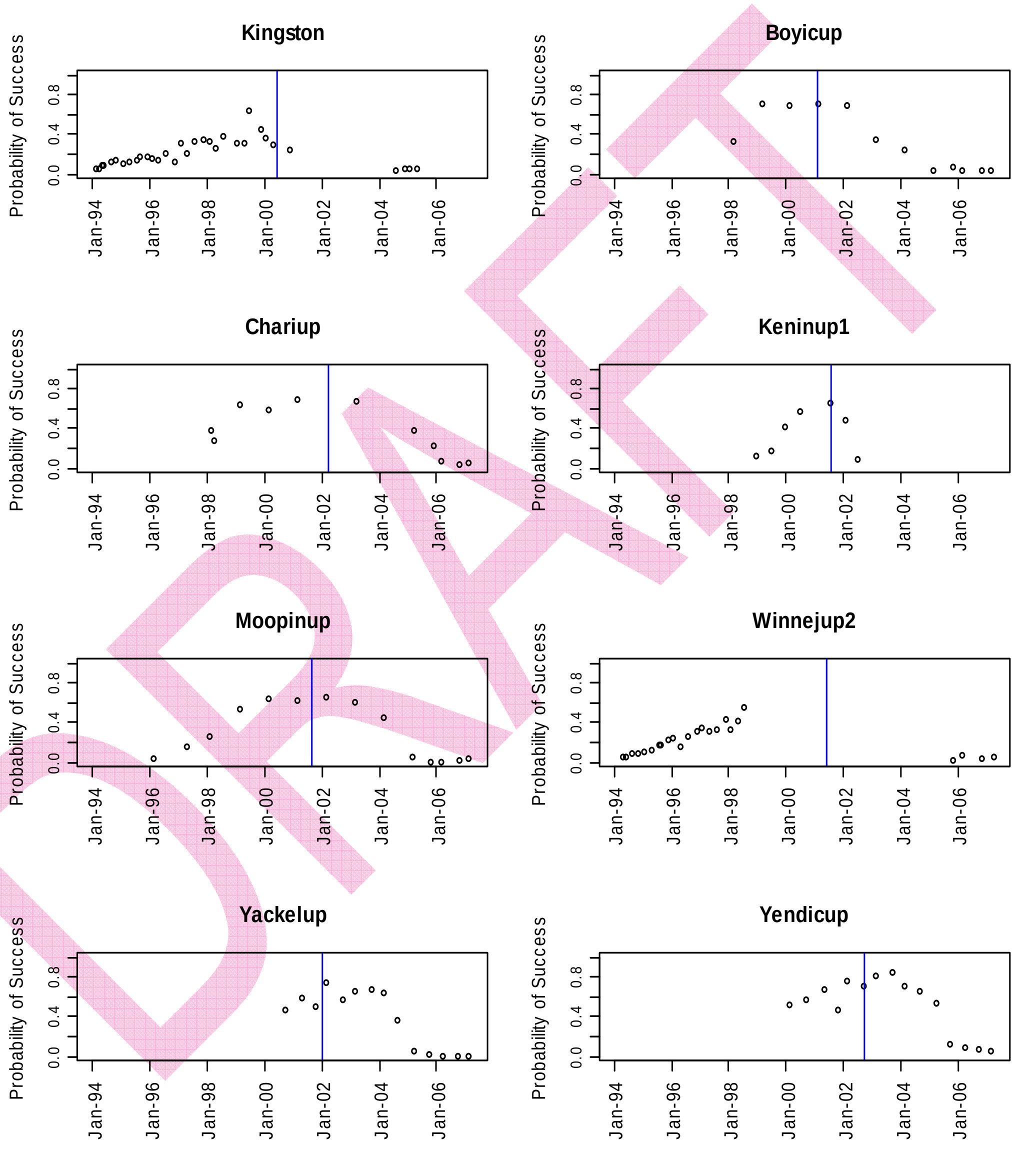

Figure 3.3.5. Probability of success (catching a woylie) over time for sites Kingston, Boyicup, Chariup, Keninup1, Moopinup, Winnejup2, Yackelup and Yendicup.

Table 3.3.9. Estimated commencement dates of decline according to model using joined sites versus visually estimated date of decline.

Site	Modelled Date of Peaking	Visually Estimated Date of Decline
Kingston	August 1999	1999
Balban	June 1999	Pre 2006
Boyicup	March 1999	2002 to 2003
Camelar	October 1997	20002 or earlier
Chariup	February 2001	2002 to 2004
Corbal	October 2001	Pre 2006
Keninup	January 2005	2001
Moopinup	November 2000	2001 to 2002
Warrup	June 2000	1998 to 2001, 2006
Winnejup	October 1999	1998 to 2006
Yackelup	April 1999	2004
Yendicup	December 2000	2004

3.3.4. Population demographics and biometrics

3.3.4.1. Sex ratio

A generalised linear model was used in identifying any changes that have occurred with regards to the sex ratio both over time and between sites. For a number of captured woylies sex wasn't recorded, therefore the dataset was restricted to those woylies where sex was recorded (approximately 95%). Success was defined as catching a male woylie and failure was defined as catching a female woylie. Male woylie individuals constituted 60% of the overall dataset. This bias in sex ratio was also evident in previous studies (Start *et al*., 1995).

Models were fitted where overlapping sites (for example Warrup1 and Warrup2) were combined into the one site (for example Warrup), however, these models showed worse fits than when the sites were left separate. Due to the significance of time variables in the model, only those sites that were retained for the earlier trend analysis were retained in the final model. The selected sites were Kingston, Boyicup, Chariup, Keninup1, Moopinup, Winnejup2, Yackelup (these were chosen as the period of data collection at these sites was long enough to exhibit an increase then a decrease in the number of woylies). This final model is shown in Table 3.3.10 and was a better fit than the previous models explored.

The explanatory variables used initially in this model consisted of various time parameters (including Time, $Time^2$, $Time^3$), site, the interaction term Time*Site, trap rate (to determine if the sex ratio is dependent on abundance), the proportion of adult woylie females that had been recorded as breeding and, a cosine and sine term (to check for any seasonal fluctuations that may be significant). The reference site was Kingston. Time, Site and the interaction between Site and Time were found to be significant. The resulting model is shown Table 3.3.10.

Table 3.3.10. Quasibinomial model where Success of Male woylies = Time+ Site+Time*Site. ('*' Pr<0.001, '**' Pr<0.01, '*' Pr<0.05)**

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	0.272	0.113	2.413	0.018	*
Time	0.001	0.002	0.595	0.553	
SiteBoyicup	-0.848	0.264	-3.211	0.002	**
SiteChariup	0.462	0.216	2.144	0.034	*
SiteKeninup1	-0.342	1.554	-0.220	0.826	
SiteMoopinup	0.587	0.252	2.334	0.021	*
SiteWinnejup2	0.004	0.148	0.024	0.981	
SiteYackelup	-0.604	0.662	-0.912	0.364	
SiteYendicup	0.423	0.403	1.050	0.296	
Time:SiteBoyicup	0.010	0.003	2.767	0.007	**
Time:SiteChariup	-0.003	0.003	-1.145	0.255	
Time:SiteKeninup1	0.007	0.019	0.393	0.695	
Time:SiteMoopinup	-0.006	0.003	-2.021	0.046	*
Time:SiteWinnejup2	-0.001	0.002	-0.231	0.818	
Time:SiteYackelup	0.009	0.006	1.404	0.163	
Time:SiteYendicup	-0.004	0.004	-0.977	0.331	

Dispersion parameter for quasibinomial family taken to be 0.5797085

Residual deviance: 65.129 on 107 degrees of freedom

The factors that were significant in explaining the sex ratio of the woylie population were:

- Sites Boyicup, Chariup and Moopinup
- The interaction between Time and the Sites Boyicup and Moopinup

Sites with a positive parameter estimate have a higher proportion of male woylies than Kingston and sites with a negative parameter estimate have a lower proportion of male woylies than Kingston.

The proportion of male woylies caught over time were found to increase at the sites Keninup, Kingston (slow increase), Winnejup (slow increase), Yackelup and Boyicup, whereas the proportion of males decreased over time for the sites Moopinup, Yendicup and Chariup.

Further analysis of this model involved looking at the validation of the model. Firstly the trap rate (defined as the number of woylies caught divided by the number of traps available) was examined with respect to the proportion of male woylies caught at these intervals. Figure 3.3.6 shows that there is no visual relationship between the trapping rate and the sex of the woylie caught (except for Camelar and Warrup2, which showed a potential slight decrease and Chariup, which showed a potential slight increase in the number of males caught as the trapping rate increased). The results shown in Figure 3.3.6 reiterate the reason why trap rate was found to be not significant.

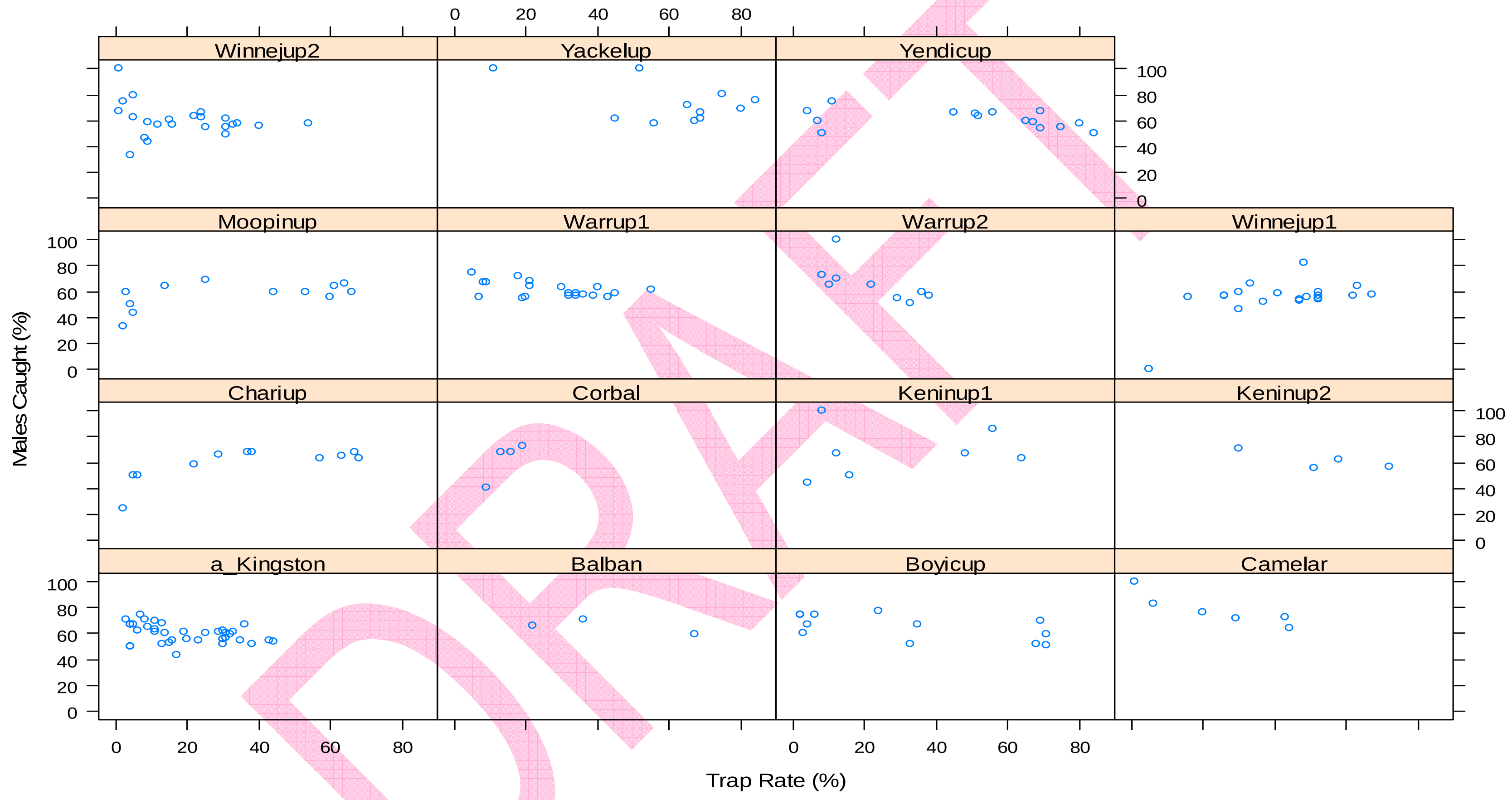

Figure 3.3.6. Percentage of males caught versus trap rate (percentage of woylies caught) for all sites.

The residuals shown in Figure 3.3.7 further emphasise the validity of the final model. The majority of the residuals only showed a slight deviation of between 0% - 20% from the observed percentages, which indicates that the model is a reasonable fit.

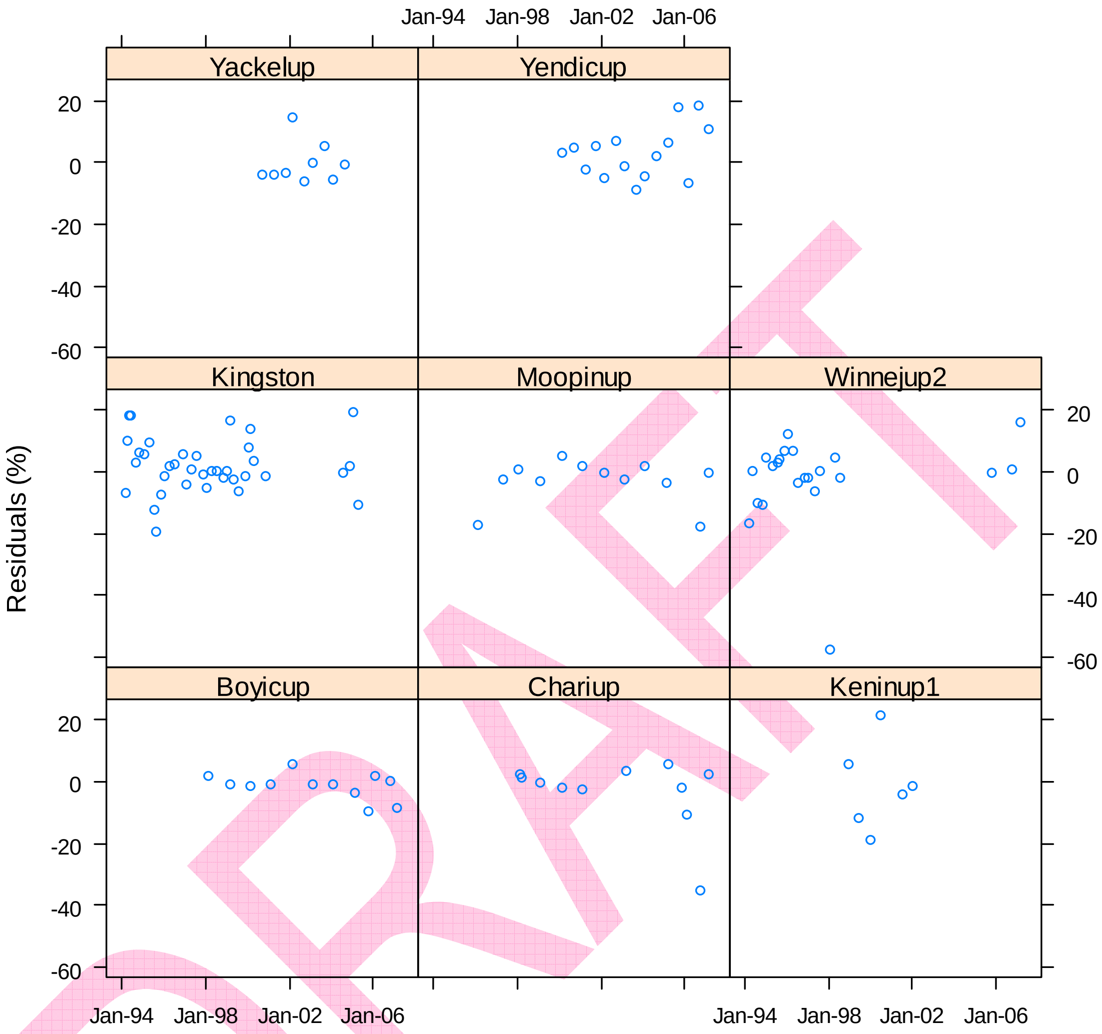

Figure 3.3.7. The residuals of the final model for each site used in the model.

3.3.4.2. Age structure

A generalised linear model was used in identifying any changes that have occurred with regards to the age structure of the woylie population both over time and between sites.

Success was defined as catching an adult woylie and failure was defined as not catching an adult woylie (i.e. the individual could be a subadult, juvenile or infant). Adult individuals made up 95% of the dataset and therefore Data Analysis Australia suggests it would be ideal if this 'Adult' category were broken down into smaller groupings, however it recognises the intrinsic difficulties in being able to do this in practice. It would however enable better evaluation of the change in age structure of the woylie population over time.

Models were tried where overlapping sites (for example Warrup1 and Warrup2) were combined into the one site (for example Warrup), these models showed worse fits than when the sites were left separate. As time was not significant in this model, the sites with a shorter period of data collection could be retained for this analysis. This final model is shown in Table 3.3.11 and, as Figure 3.3.8 suggests, the model is of reasonable fit.

Again, other explanatory variables were introduced into the model such as cosine and sine terms to model any possible seasonal variation in the data, trap rate, the proportion of adult woylie females that had been recorded as breeding and various time parameters. However these were found to be not significant. Hence, Site was the only factor that was retained in the final model.

Table 3.3.11. Quasibinomial model where Success of Adults = Site. ('*' Pr<0.001, '**' Pr<0.01, '*' Pr<0.05)**

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	2.508	0.165	15.245	<2.00E-16	***
SiteBalban	0.558	0.382	1.460	0.147	
SiteBoyicup	0.854	0.356	2.400	0.018	*
SiteCamelar	0.026	0.342	0.075	0.940	
SiteChariup	0.371	0.283	1.313	0.192	
SiteCorbal	0.354	0.598	0.592	0.555	
SiteKeninup1	0.057	1.172	0.049	0.961	
SiteKeninup2	-0.331	0.261	-1.267	0.208	
SiteMoopinup	0.715	0.294	2.434	0.017	*
SiteWarrup1	-0.474	0.197	-2.409	0.018	*
SiteWarrup2	-0.211	0.329	-0.643	0.522	
SiteWinnejup1	-0.248	0.218	-1.136	0.259	
SiteWinnejup2	-0.247	0.254	-0.972	0.333	
SiteYackelup	0.123	0.319	0.386	0.700	
SiteYendicup	0.044	0.319	0.138	0.891	

Dispersion parameter for quasibinomial family taken to be 1.485833

Residual deviance: 139.67 on 103 degrees of freedom

The sites that were found to be significant were: Boyicup, Moopinup and Warrup1. Sites with a positive parameter estimate have a higher proportion of adult woylies than Kingston and sites with a negative parameter estimate have a lower proportion of adult woylies than Kingston. There was an indication that more adult woylies were caught at Balban, Boyicup, Camelar, Chariup, Corbal, Keninup1, Moopinup, Yackelup and Yendicup and less at Keninup2, Warrup1, Warrup2, Winnejup1 and Winnejup2.

Further analysis of this model involved looking at the trap rate (defined as the number of woylies caught divided by the number of traps available). This was examined with respect to the proportion of adult woylies caught at these intervals. Figure 3.3.9 shows that there is no visible relationship between the trapping rate and the age of the woylie caught. The results shown in Figure 3.3.9 reiterate the reason why trap rate was found to be not significant.

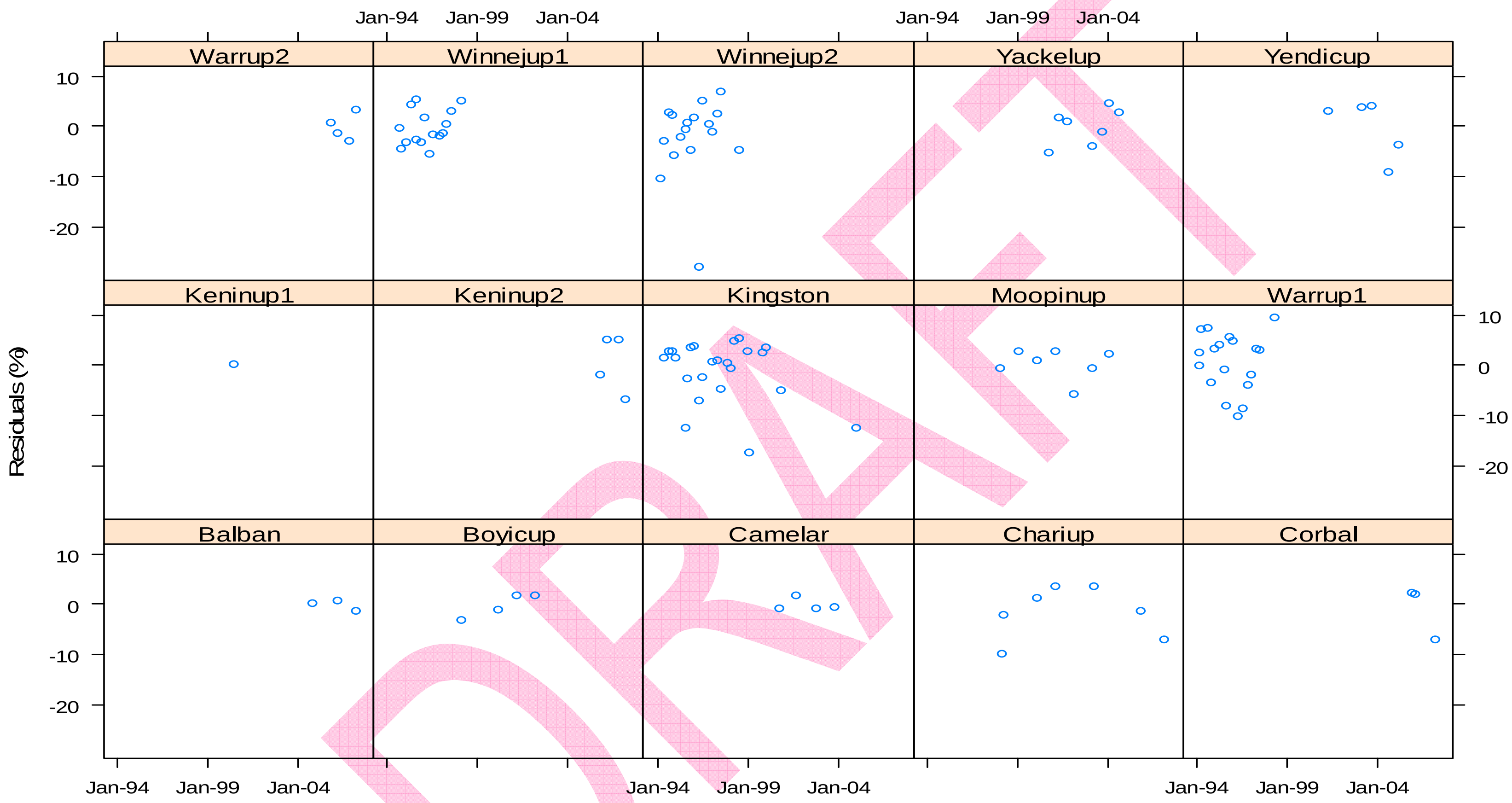

Figure 3.3.8. The residuals of the final model for each site used in the model plotted against time.

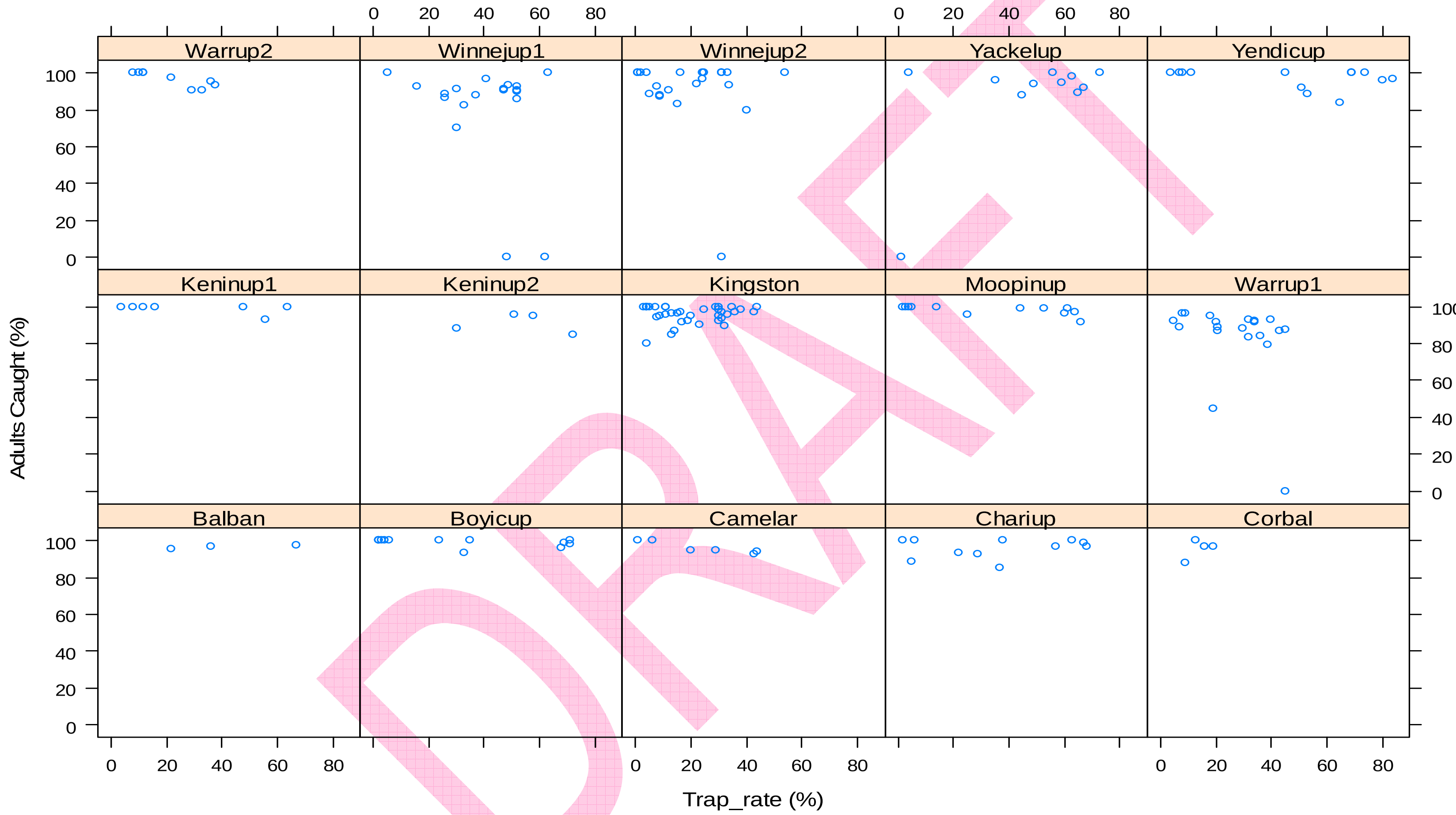

Figure 3.3.9. Percentage of adults caught versus trap rate (percentage of woylies caught) for all sites.

3.3.4.3. Weight

A linear model was used to identify any changes in the weights recorded for woylies caught since January 1994 for all sites. All sites were incorporated into the model initially, however due to the significance of the Time/Site interaction the sites were then selected on the basis of their underlying quadratic nature.

The explanatory variables used in the initial model were Time, $Time^2$, $Time^3$, Site, Age, Sex, Trap Rate, the proportion of adult woylie females that had been recorded as breeding and the interaction term between Time and Site. Other explanatory variables were introduced into the model such as cosine and sine terms to model any possible seasonal variation in the data. The resulting model is shown below in Table 3.3.12.

The reference group included the site Kingston, female woylie and adult woylie.

Table 3.3.12. Linear model where Weight = Cosine term + Sine term + Site + Age + Sex + Time*Site. ('*' Pr<0.001, '**' Pr<0.01, '*' Pr<0.05)**

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	1330.199	9.385	141.73	2.07E-03	***
Infant	-1271.597	46.346	-27.44	2.07E-03	***
Juvenile	-1055.401	14.564	-72.46	2.07E-03	***
Subadult	-411.082	17.438	-23.58	2.07E-03	***
Unknown Age	-44.786	14.531	-3.08	2.07E-03	**
Male	-20.797	3.888	-5.35	9.22E-08	***
Unknown Sex	-52.663	23.736	-2.22	0.03	*
SiteBoyicup	-160.095	20.979	-7.63	2.79E-14	***
SiteChariup	-70.710	17.432	-4.06	5.06E-05	***
SiteKeninup1	469.030	121.637	3.86	1.17E-04	***
SiteMoopinup	51.483	19.642	2.62	0.01	**
SiteWinnejup2	-5.723	13.870	-0.41	0.68	
SiteYackelup	-284.559	52.393	-5.43	5.87E-08	***
SiteYendicup	45.153	48.922	0.92	0.36	
Time	0.221	0.163	1.36	0.17	
cosine_term	12.005	3.905	3.07	2.12E-03	**
sine_term	5.804	3.377	1.72	0.09	.
SiteBoyicup:Time	1.692	0.262	6.45	1.20E-10	***
SiteChariup:Time	0.932	0.222	4.21	2.61E-05	***
SiteKeninup1:Time	-4.707	1.433	-3.28	1.03E-03	**
SiteMoopinup:Time	-0.393	0.246	-1.60	0.11	
SiteWinnejup2:Time	1.069	0.298	3.59	3.40E-04	***
SiteYackelup:Time	2.888	0.507	5.69	1.31E-08	***
SiteYendicup:Time	-0.630	0.447	-1.41	0.16	

Residual standard error: 130.2 on 4788 degrees of freedom
Multiple R-Squared: 0.6014, Adjusted R-squared: 0.5995
F-statistic: 314 on 23 and 4788 DF, p-value: < 2.2e-16

The factors that are significant when looking at changes in the weight of woylies are:

- All age parameters
- All sex parameters
- The cosine term (therefore seasonality is a contributing factor)
- The sites Boyicup, Chariup, Keninup1, Moopinup and Yackelup

- The interaction between Time and the sites Boyicup, Chariup, Keninup1, Winnejup2 and Yackelup

In general, female adult woylies were found to be heavier than male adult woylies, however, woylies of unknown sex were found to be even lighter. The weights of woylies differed between sites. The weights of woylies at the northeastern sites (Keninup, Moopinup and Yendicup) at the beginning of the study were heavier than those individuals found at Kingston (reference group), however, the weights at these sites have decreased over time relative to Kingston. The southeastern sites (Boyicup, Chariup and Yackelup) were less weighty than those individuals found at Kingston, however these woylies have increased in weight over time relative to Kingston individuals.

Further analysis of model included examining the residuals (dispalyed in Figure 3.3.10). The residuals show that the model is a reasonable fit to the dataset, with the majority of the residuals lying between ± 500 grams of the 'actual' data points.

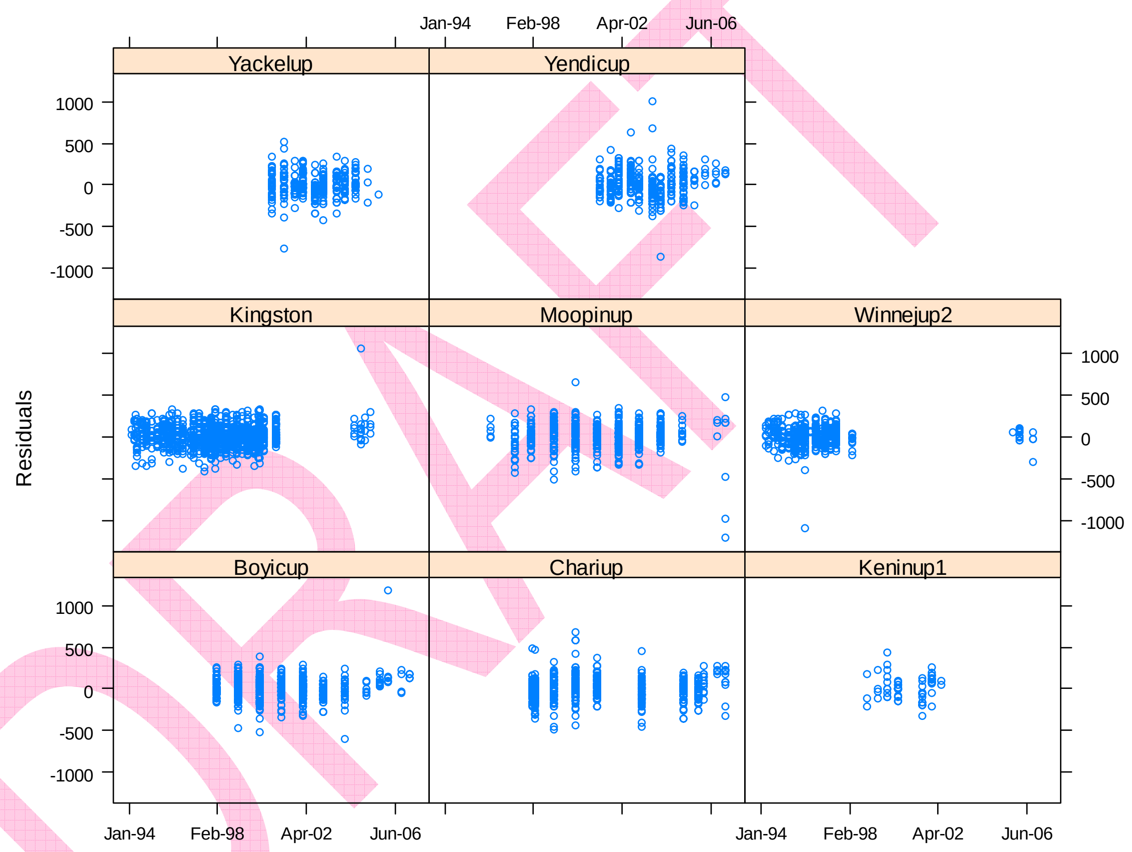

Figure 3.3.10. The residuals of the final model for each site used in the model plotted against time.

Further analysis of this model involved looking at the trap rate (defined as the number of woylies caught divided by the number of traps available). This was examined with respect to the weight of the woylies caught at these intervals. Figure 3.3.11 shows that there is no visible relationship between the trapping rate and the weight of the woylie caught. The results shown in Figure 3.3.11 reiterate the reason why trap rate was found to be not significant.

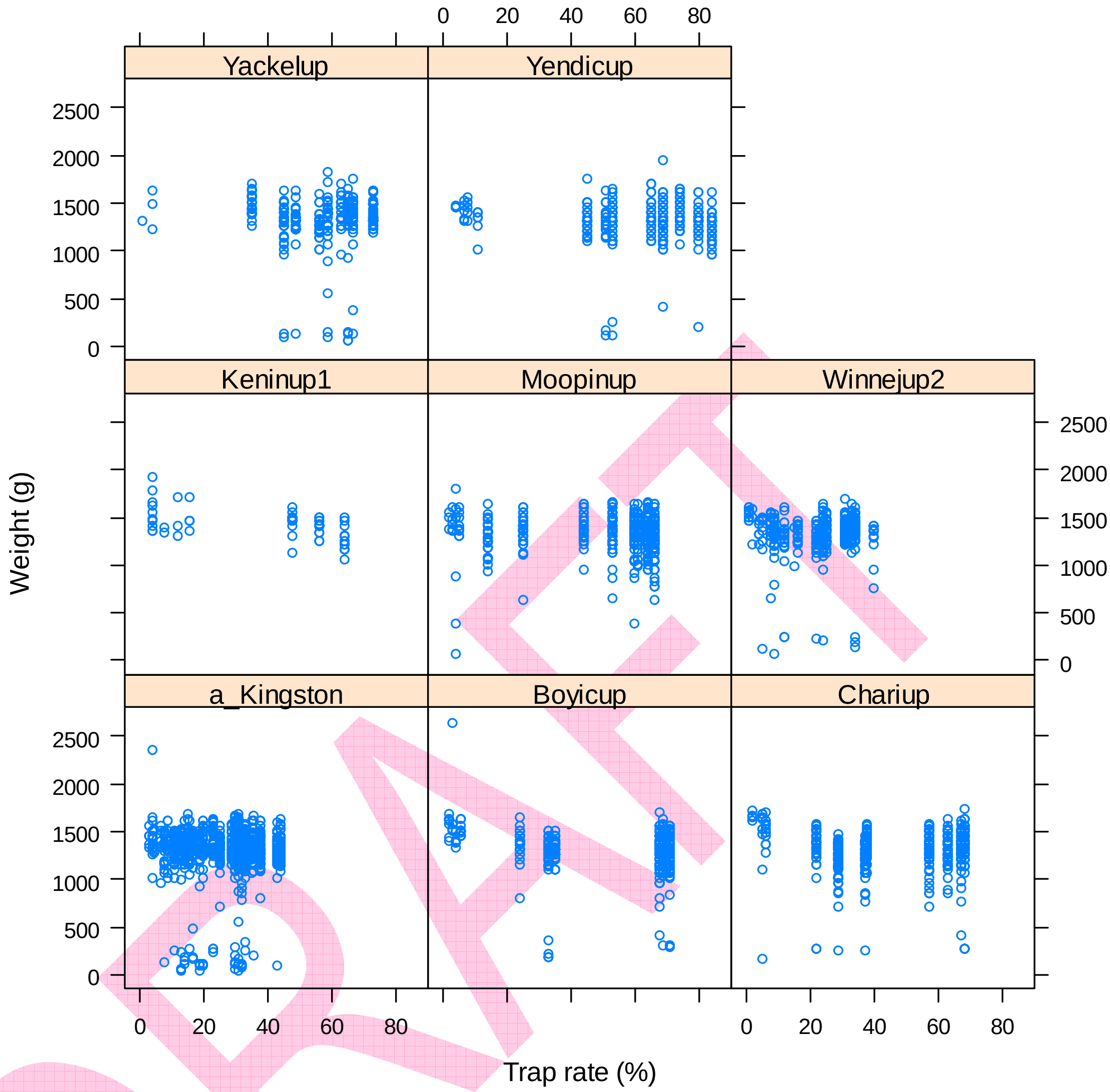

Figure 3.3.11. Weight versus trap rate (percentage of woylies caught) for all sites.

3.3.4.4. Hindfoot length

A linear model was used to identify any changes in hindfoot length recorded for individual woylies caught since January 1994 for all sites. Again, the reference group included Kingston, female woylie and adult woylie.

All sites were incorporated into the model initially, however due to the significance of the Time/Site interaction the sites were then selected on the basis of their underlying quadratic nature.

The explanatory variables used in the initial model were Time, Site, Age, Sex, and the interaction term between Time and Site. Other explanatory variables were introduced into the model such as cosine and sine terms to model any possible seasonal variation in the data, $Time^2$, $Time^3$, Trap Rate, the proportion of adult woylie females that had been recorded as breeding and the Time/Sex interaction. However, these variables were found to be not significant. The final model is shown in Table 3.3.13.

Table 3.3.13. Linear model where Hindfoot Length = Time + Site + Age + Sex + Time*Site. ('*' Pr<0.001, **' Pr<0.01, '*' Pr<0.05)**

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	107.847	0.374	288.29	<2.00E-16	***
Infant	-56.878	1.943	-29.27	< .00E-16	***
Juvenile	-21.586	0.651	-33.14	< .00E-16	***
Subadult	-2.824	0.835	-3.38	7.27E-04	***
Unknown Age	-1.507	1.470	-1.03	0.31	
Male	0.920	0.211	4.36	1.35E-05	***
Unknown Sex	-3.296	2.911	-1.13	0.26	
Time	0.007	0.006	1.17	0.24	
SiteBoyicup	-5.466	1.007	-5.43	6.32E-08	***
SiteChariup	-4.235	0.758	-5.58	2.67E-08	***
SiteKeninup1	2.617	5.354	0.49	0.63	
SiteMoopinup	0.449	1.015	0.44	0.66	
SiteWinnejup2	-0.147	0.563	-0.26	0.79	
SiteYendicup	64.786	73.125	0.89	0.38	
Time:SiteBoyicup	0.040	0.014	2.80	5.24E-03	**
Time:SiteChariup	0.043	0.011	4.05	5.26E-05	***
Time:SiteKeninup1	-0.023	0.066	-0.35	0.72	
Time:SiteMoopinup	-0.017	0.015	-1.11	0.27	
Time:SiteWinnejup2	0.013	0.011	1.15	0.25	
Time:SiteYendicup	-0.428	0.471	-0.91	0.36	

Residual standard error: 4.732 on 2095 degrees of freedom

Multiple R-Squared: 0.5033, Adjusted R-squared: 0.4988

F-statistic: 111.7 on 19 and 2095 DF, p-value: < 2.2e-16

The factors that are significant when looking at changes in the hindfoot length of woylies are:

- The age parameters Infant, Juvenile and Subadult
- The sex parameter Male
- Sites Boyicup and Chariup
- The interaction between Time and the sites Boyicup and Chariup

Male adult woylies have a larger hind foot length than female adult woylies and tended to be larger in the same sites where the weights were found to be heavier.

Further analysis was conducted of the hindfoot length with regards to the residuals produced by this model. Figure 3.3.12 indicates that the model is a good fit to the data as the majority of the residuals show a ±30mm difference between the fitted values of the model and the 'actual' data. Yackelup is missing due to the absence of hindfoot length measurements in the dataset.

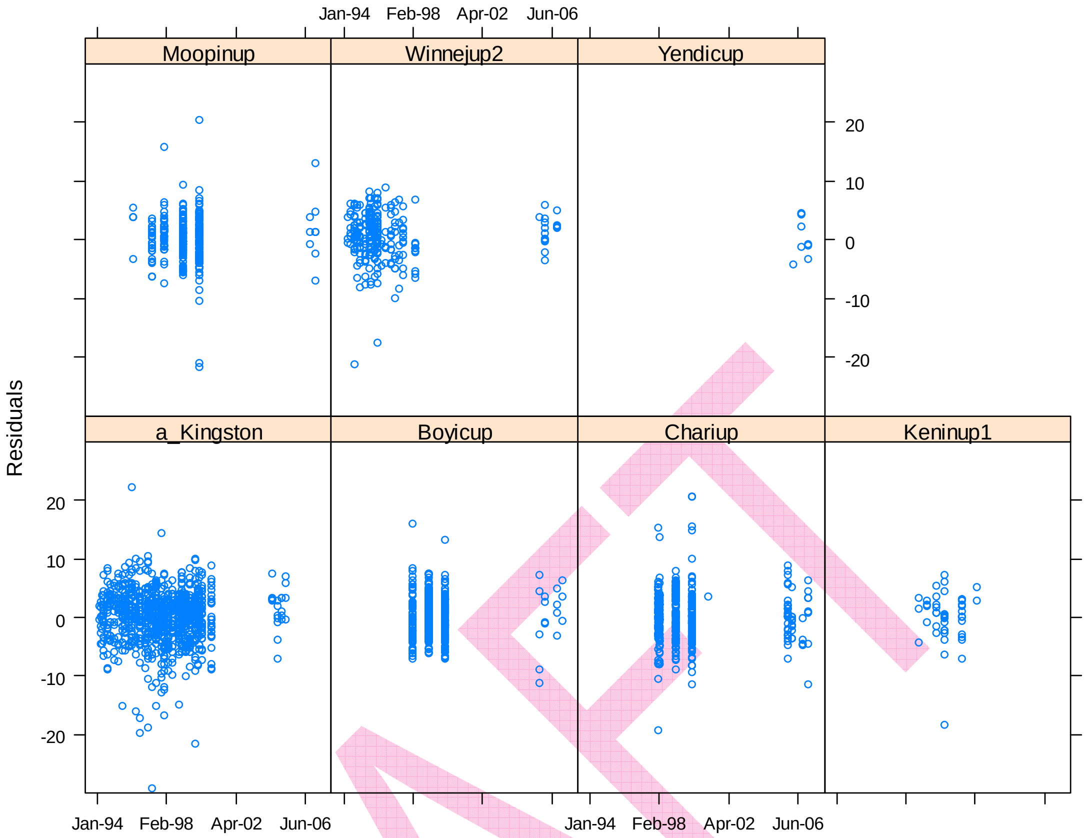

Figure 3.3.12. The residuals of the final model for each site used in the model plotted against time.

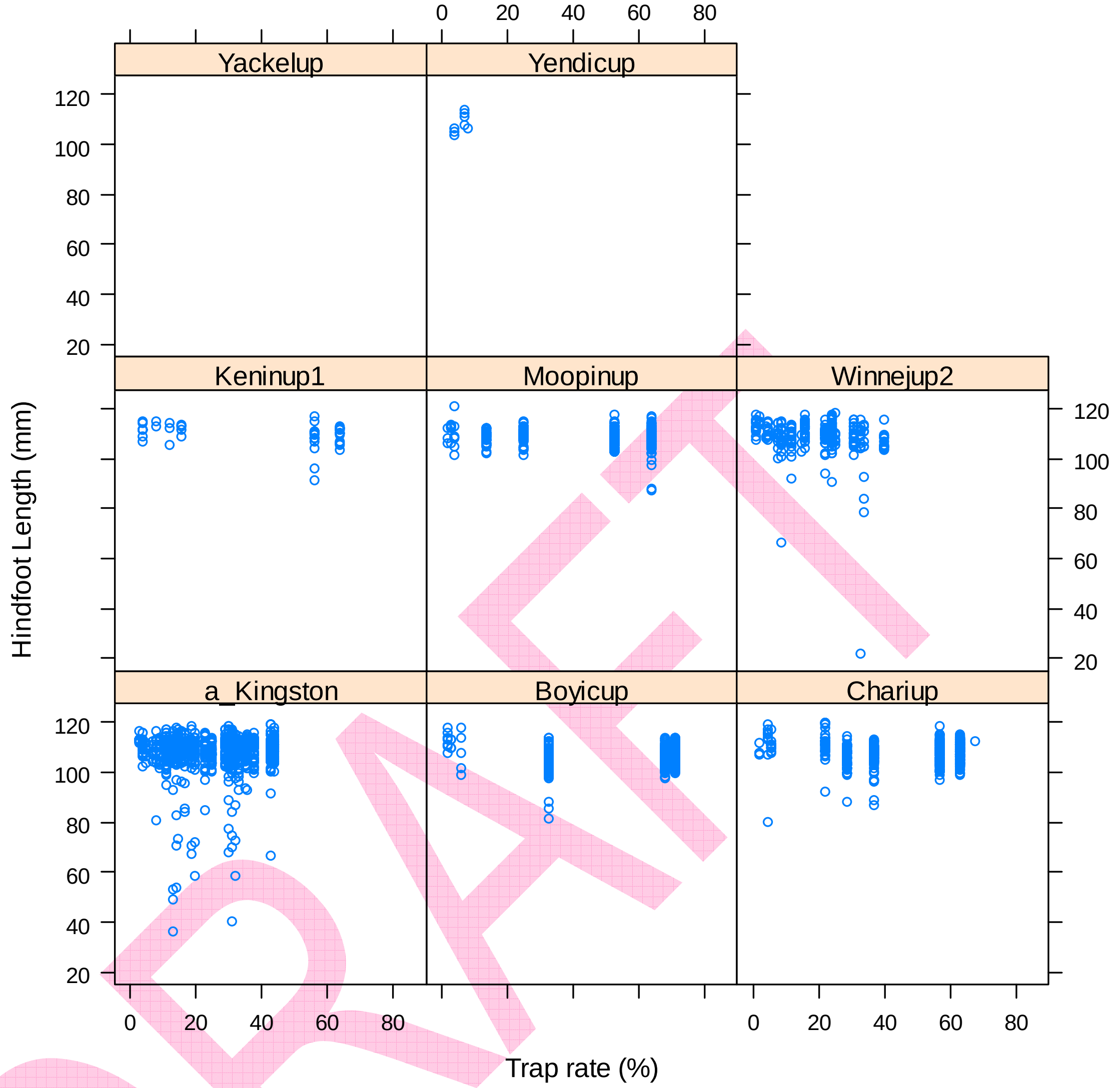

Figure 3.3.13. Hindfoot length versus trap rate (percentage of woylies caught) for all sites.

3.3.4.5. Head length

A linear model was used to identify any changes in head length recorded for woylies caught since January 1994 for all sites. Again, the reference group included Kingston, female woylie and adult woylie.

The explanatory variables used in the initial model were Time, $Time^2$, $Time^3$, Site, Time, Age, Sex, Trap Rate and the interaction terms Time*Site and Time*Sex.

Other explanatory variables were introduced into the model such as the proportion of adult woylie females that had been recorded as breeding, and, cosine and sine terms to model any possible seasonal variation in the data. However these were not found to be significant.

All sites were included in this model, however in all models explored the sites themselves were found to be not significant regardless of whether they were combined, separated or selected on the basis of their underlying quadratic nature. The resulting model is shown in Table 3.3.14.

Table 3.3.1. Linear model where Head Length = Age. ('*' Pr<0.001, '**' Pr<0.01, '*' Pr<0.05)**

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	84.46489	0.08297	1018.051	<2.00E-16	***
Infant	-46.1649	1.31379	-35.139	<2.00E-16	***
Juvenile	-29.1456	0.49817	-58.506	<2.00E-16	***
Subadult	-6.54065	0.65089	-10.049	<2.00E-16	***
Unknown Age	-3.53156	1.07378	-3.289	0.001	**

Residual standard error: 3.709 on 2103 degrees of freedom

Multiple R-Squared: 0.6906, Adjusted R-squared: 0.69

F-statistic: 1173 on 4 and 2103 DF, p-value: < 2.2e-16

All age parameters were found to be significant in the model.

Further examination of the residuals is shown in Figure 3.3.14. This indicates that the model is a good fit to the data as the majority of the residuals show a ± 20mm difference between the fitted values of the model and the 'actual' data. Yackelup is missing due to the absence of head length measurements in the dataset for this site.

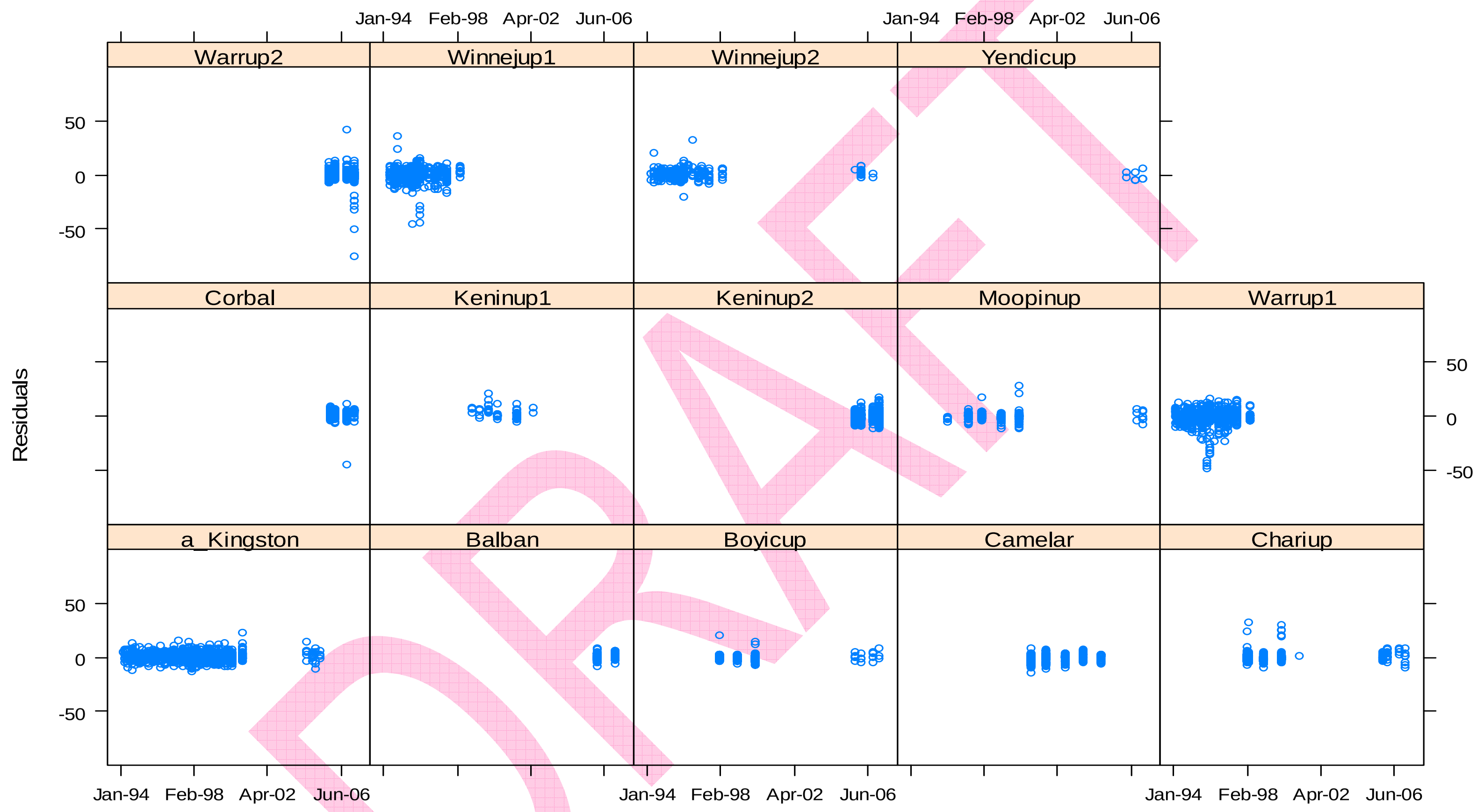

Figure 3.3.14. The residuals of the final model for each site used in the model plotted against time

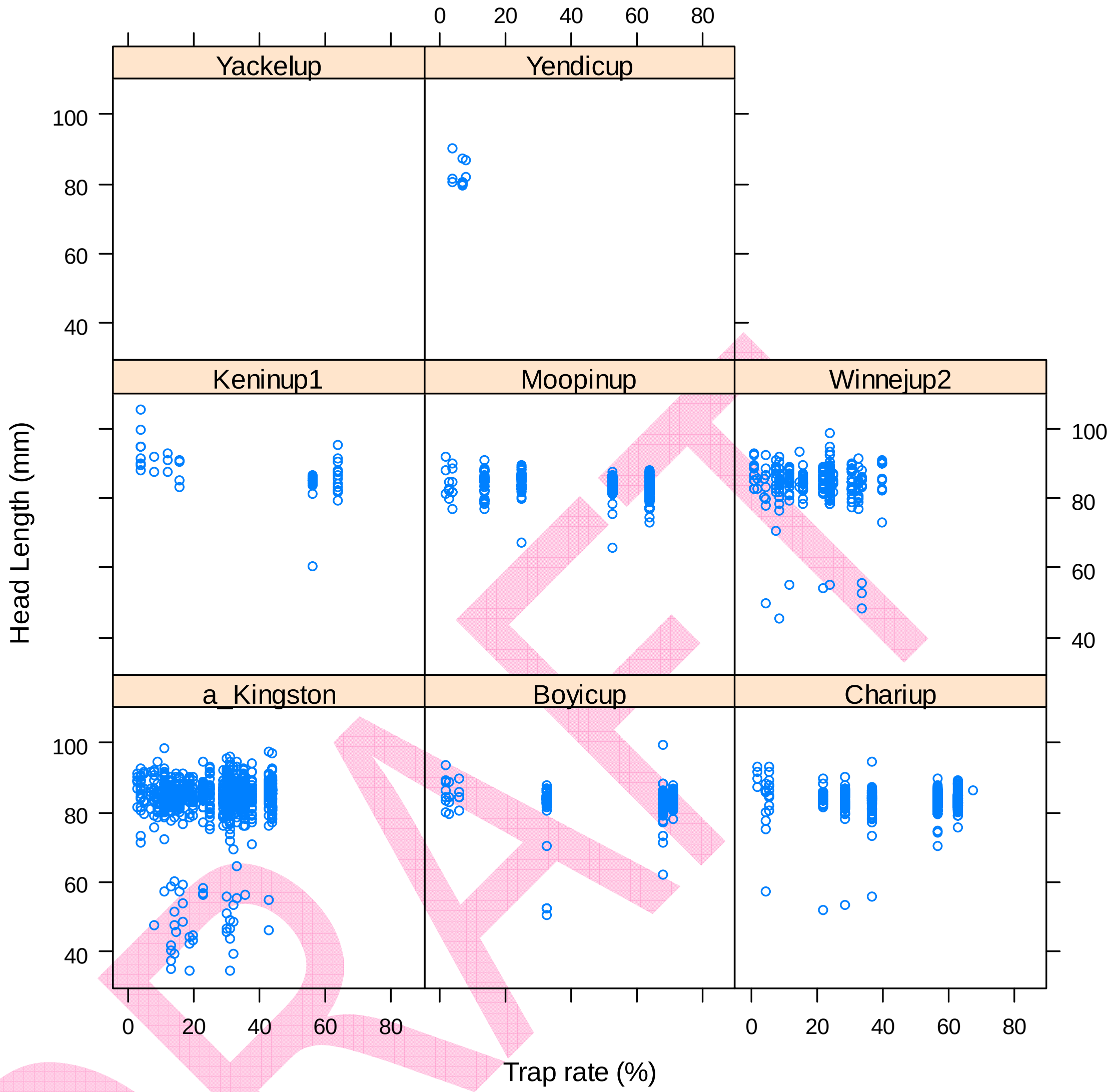

Figure 3.3.15. Head length versus trap rate (percentage of woylies caught) for all sites.

3.3.4.6. Condition index

The condition index was used in a linear model to assess whether the body condition of adult woylie individuals has changed with regards to time, seasonality (cosine and sine terms), site and sex of the woylie individual. Sites were selected on the basis of their underlying quadratic nature.

The explanatory variables used in the initial model were Time, Time^2, Time^3, Site, Sex, cosine term, sine term, Time*Site and Site*Sex. This model showed some evidence of an increase in condition index for the sites Kingston, Boyicup, Chariup, Winnejup2 and Yendicup and a decrease in condition index for the sites Keninup1 and Moopinup. However, given the variation in the condition index of individuals no reasonable model could be formulated to predict the condition index of an adult woylie.

3.3.4.7. Breeding adult female woylies

The definition of a breeding woylie was outlined in Section 3.3.2.1. An exploratory plot of the proportion of breeding female woylies over time (Figure 3.3.16) did not show any visual relationship for the sites Kingston, Boyicup, Chariup Warrup1, Keninup2, Keninup1 and Winnejup1. The sites Warrup2, Winnejup2, Yackelup, Corbal, Moopinup, Camelar and Balban showed some indication of a possible increase or decrease in the proportion of breeding adult female woylies over time.

The proportion of breeding adult female woylies was then modelled using a generalised linear model to assess if there were any trends over time, differences between sites and any relationships with demographic and biometric variables. Success was defined as an adult female woylie that was recorded as breeding and failure was defined as an adult female woylie that was not recorded as breeding.

A number of models were attempted to fit the data however the models generated were not reliable or conclusive in showing trends over time. It was found that the different models gave vastly different results, the dispersion parameters were extreme and the residuals showed poor fits. This indicates that any model produced using time, demographic or biometric variables would not be robust and may lead to incorrect conclusions being drawn. The only consistent result was that site differences were always evident. This inability to fit a reliable model in which one could be confident in the results is not surprising considering the difficulties involved in calculating accurate measures of the breeding rates as discussed in Section 3.3.2.1.

As a site difference was evident a simplified model using site only as an explanatory variable was fitted and gave a strong indication that there are differences in breeding rates between sites. However, this model should only be used to provide an idea of differences between sites and should not be used in predicting the proportion of adult female woylies that are breeding. The results are shown in Table 3.3.15 and the reference site was Kingston.

Table 3.3.15. Quasibinomial model where Success of Breeding = Site. ('*' Pr<0.001, '**' Pr<0.01, '*' Pr<0.05)**

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	0.283	0.027	10.390	<2.00E-16	***
SiteBoyicup	-0.754	0.038	-19.898	<2.00E-16	***
SiteChariup	-0.951	0.041	-23.084	<2.00E-16	***
SiteKeninup1	1.145	0.393	2.914	0.004	**
SiteMoopinup	-1.154	0.041	-28.479	<2.00E-16	***
SiteWinnejup2	0.389	0.063	6.201	6.05E-10	***
SiteYackelup	1.019	0.091	11.171	<2.00E-16	***
SiteYendicup	1.231	0.072	17.173	<2.00E-16	***

Dispersion parameter for quasibinomial family taken to be 5.823072

Residual deviance: 33275 on 5222 degrees of freedom

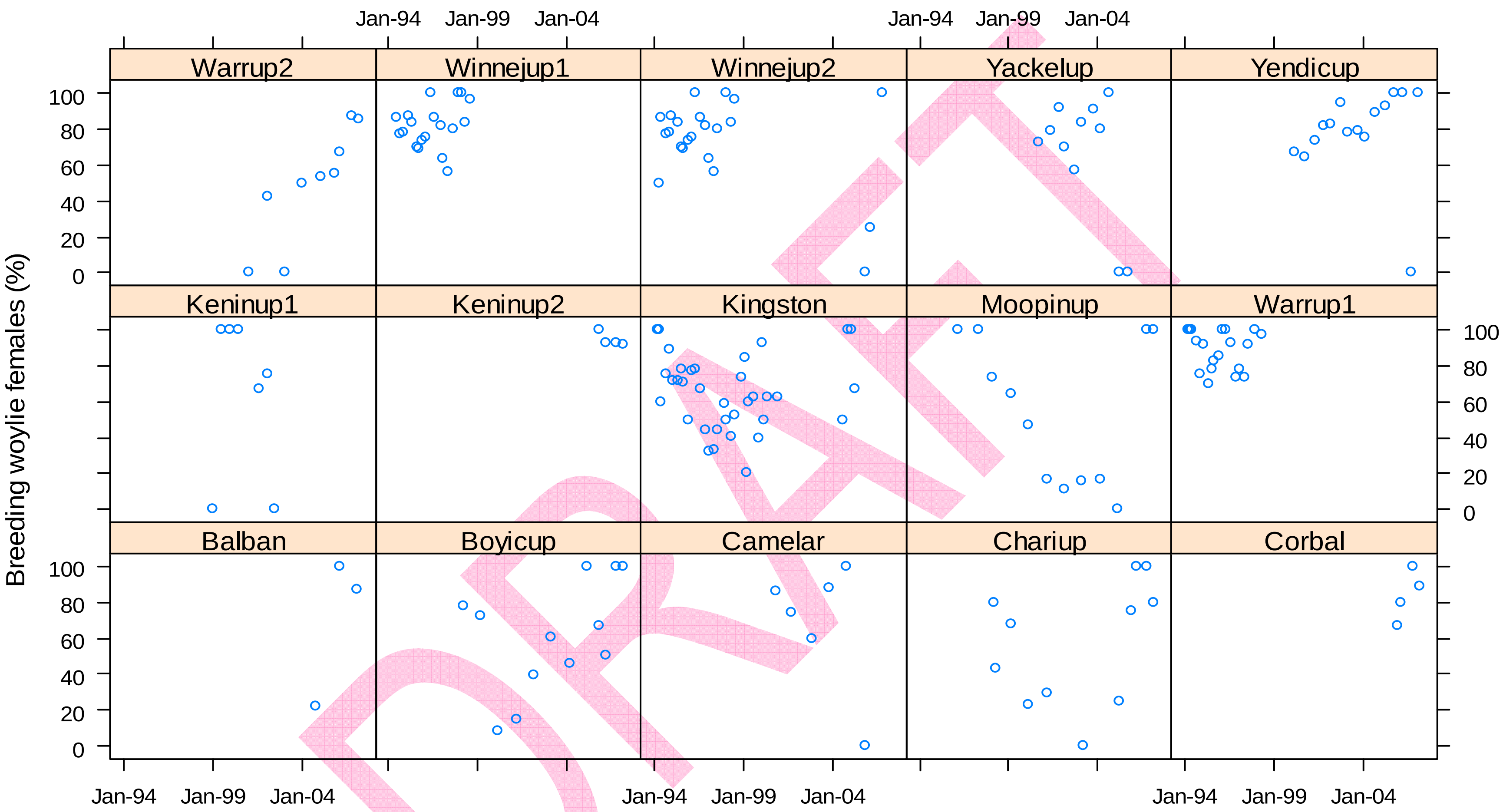

Figure 3.3.16. Percentage of female adult woylies that are breeding over time for all sites.

3.3.5. Conclusions

Population change was modelled via a generalised linear model. The high dispersion shown for each of the models produced demonstrates that the use of a quasibinomial family was the most appropriate in producing these models. Multiple models were investigated and showed similar results, which verified the robustness of the models used in assessing population change.

One of the key questions asked in this analysis was "what can be objectively said regarding the decline (or otherwise) of the woylie population?" The answer is provided in the model shown in Table 3.3.7. The highly statistically significant $Time^3$ term models the strong decline (shown in Figure 3.3.3) that has occurred in the woylie population since 2002. This model is a reasonable approximation of the past twelve years of woylie trapping data. Assuming trapping data is a good indicator of woylie population, this model provides strong evidence of a major decline in the woylie population since 2002.

Any associations the woylies have with other species were not definitive. Quenda were shown to be significant (a positive association) in explaining the capture of a woylie yet common brushtail possums were only barely significant (again positive) and chuditch were not found to be significant.

The average condition index showed an increasing trend in the sites Boyicup, Chariup, Kingston and Yendicup. This increase in condition index was further emphasised when the condition index of individuals were modelled against time and other variables. In the population decline model, the average condition index was fitted as an explanatory variable and was found to be not significant in explaining the decline. This suggests that the decline is not influenced by the body condition of the woylie population.

More male woylies were caught over the duration of the study with approximately 60% of individuals caught being male. The proportion of male woylies caught over time were found to increase at the sites Keninup, Kingston (slow increase), Winnejup (slow increase), Yackelup and Boyicup, whereas the proportion of males decreased over time for the sites Moopinup, Yendicup and Chariup.

The majority of the woylies caught were adults (95%) which restricted the analysis that could take place with respect to the age of the woylie individuals. Using Kingston as a reference group the proportion of adult woylies was investigated at each site. . There was an indication that more adult woylies were caught at Balban, Boyicup, Camelar, Chariup, Corbal, Keninup1, Moopinup, Yackelup and Yendicup and less at Keninup2, Warrup1, Warrup2, Winnejup1 and Winnejup2.

In general, female adult woylies were found to be heavier than male adult woylies, however, woylies of unknown sex were found to be even lighter. The weights of woylies differed between sites. The weights of woylies at the northeastern sites (Keninup, Moopinup and Yendicup) at the beginning of the study were heavier than those individuals found at Kingston (reference group), however, the weights at these sites have decreased over time relative to Kingston. The southeastern sites (Boyicup, Chariup and Yackelup) were less weighty than those individuals found at Kingston, however these woylies have increased in weight over time relative to Kingston individuals.

Other measures of size used in the models were hind foot length and head length. Male adult woylies have a larger hind foot length than female adult woylies and tended to be larger in the same sites where the weights were found to be heavier. Head length was found to be affected by age only.

Investigation was conducted into whether trap capture rates were related to woylie biometrics and demographics (including proportion of adult females in breeding condition), however, no significant statistical relationship was found. Differences were found between the proportion of breeding adult female woylies at different sites, however, no other conclusive relationships could be found with time, demographic or biometric variables with regards to the proportion of breeding adult female woylies.

3.3.6. References

Start, T., A. Burbidge and D. Armstrong. 1995. Woylie Recovery Plan: Wildlife Management Program No 16. Department of Conservation and Land Management, South Australian Department of Environment and Natural Resources, and Australian National Parks and Wildlife Service.

3.4. Population trends – spatial patterns of woylie decline

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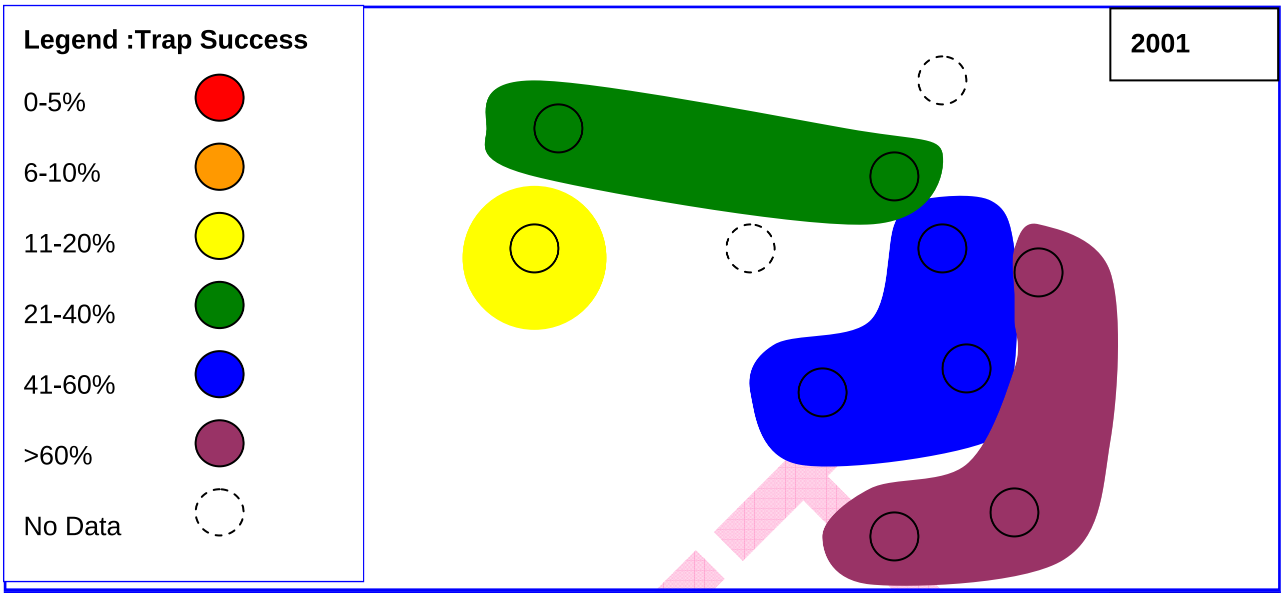
Spatial characterisation of the woylie declines is considered an important form of evidence that may be particularly powerful in assisting in the identification of key agents of decline. A rigorous spatial analysis has not yet been conducted. Expert assistance in spatial analysis is being sought to assist with this.

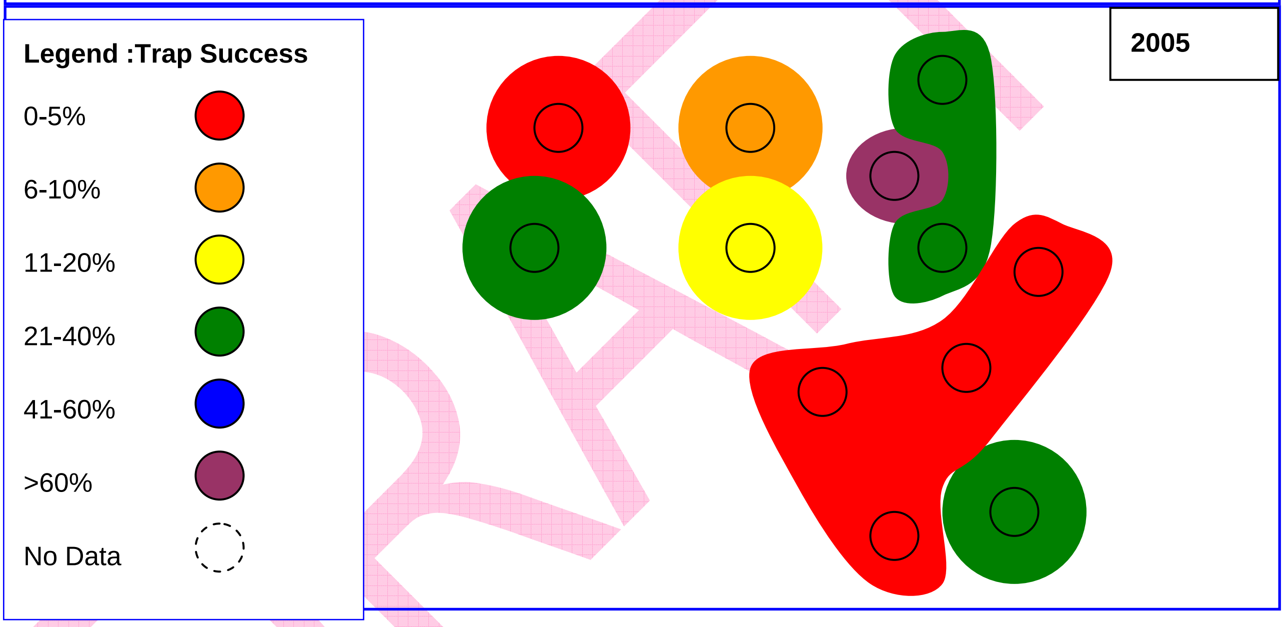
A superficial exploration of the data reveals the potential for a striking pattern to the declines. There appears to be a spatial progression of the decline, indicative of a front progressing through the region. The rate of spread appears somewhat limited, which if substantiated, could provide evidence that the agent(s) of decline may have some limited mobility.

Three central sites (Moopinup, Yackelup and Camelar) were the first sites to repeatedly catch no woylies, where previously high capture rates had been observed. The Perup Ecology Centre is approximately central to these sites (i.e. potentially "ground zero"). The declines roughly expanded outwards from this area, first to the south and then to the north. Winnejup is the exception to this pattern, declining relatively sooner than would be expected based on a declining radiation from a single point. Based on the patterns observed, the declines at Balban were predicted to occur in 2006/2007, 6-12 month before they happened. The opportunity of the anticipated declines was realised by incorporating this site in the PCS study (Chapter 4). It is also predicted on the same basis that Keninup will undergo a similar decline in 2008.

In some cases the declines began after the capture rates of woylies were particularly high. It is, therefore, also possible that there may a density-dependency relationship associated with the declines.

A more rigorous analysis of the spatial, temporal and density characteristics of the declines are clearly needed to establish whether the speculation derived from anecdotal observations can be verified. In the meantime, Figure 3.4.1 provides a crude and preliminary attempt to graphically depict the spatial and temporal pattern of the declines within the Upper Warren region. This does not however, effectively show the complete and true nature of the patterns of declines.


Legend :Trap Success
0-5%
6-10%
11-20%
21-40%
41-60%
>60%
No Data
2001


Legend :Trap Success
0-5%
6-10%
11-20%
21-40%
41-60%
>60%
No Data
2005

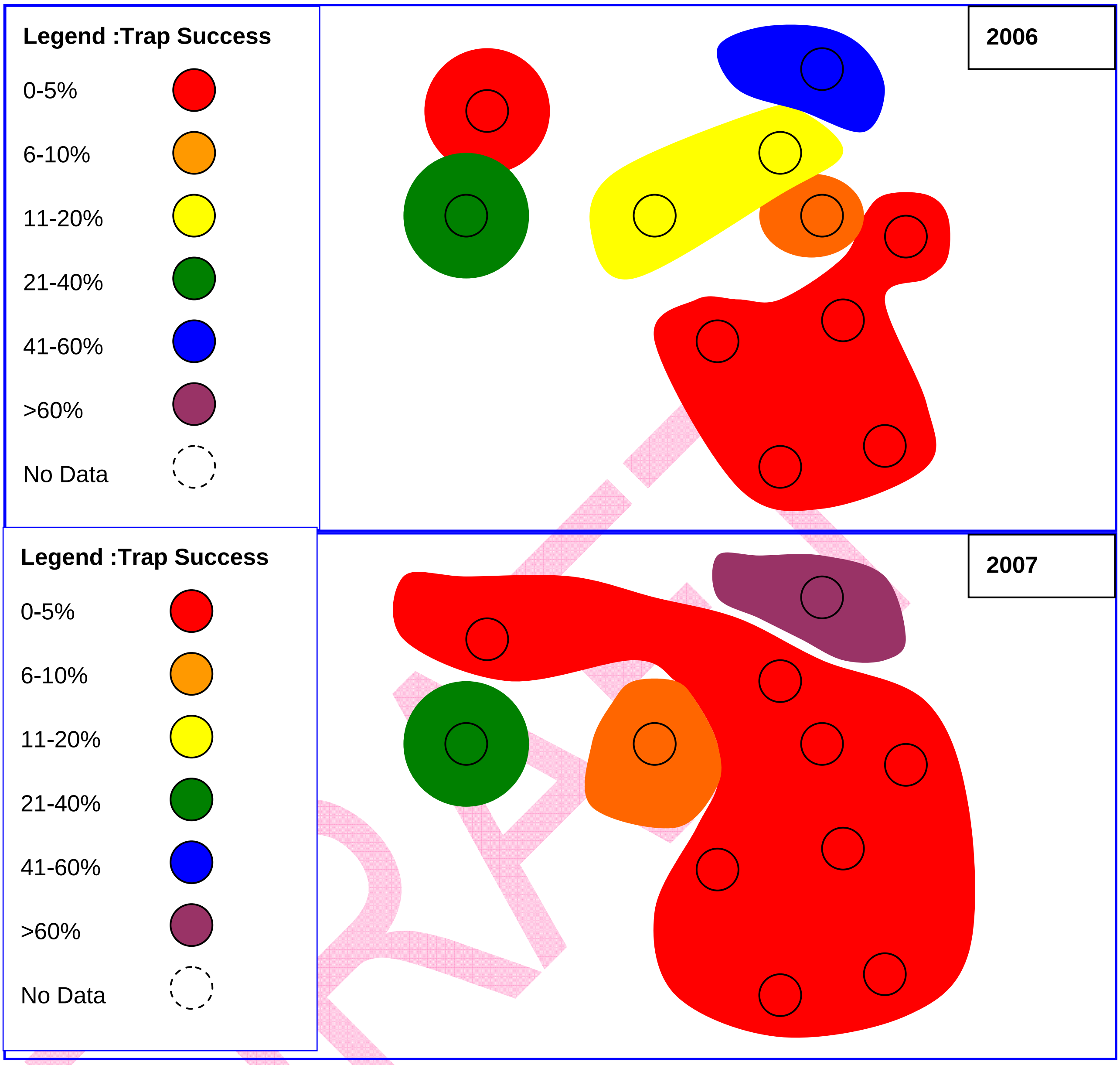


Figure 3.4.1. Rough depiction of the spatial and temporal characteristics of the woylie decline in the Upper Warren region (2001 - 2007). Circles symbolise the Upper Warren Fauna Monitoring trapping transects used to measure the trap success of woylies.

3.5. Direct human interference

Julia Wayne and Ian Wilson

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Abstract

Direct human interference was recognized as one of the potential factors influencing the decline of woylies in southwestern Australia.

Direct human interference covers a wide range of activities and factors including; trapping intensity, live harvest for translocation, trapping consequences (deaths, predations and pouch young intervention), illegal killing/harvesting, and road kills. This report focuses on trapping activities.

Despite the potential for significant impact, the results indicate that, in the Upper Warren region, human interference has not been a major contributing factor in the decline of woylie populations.

It is, however, important to continue to monitor these potential impacts through adequate and comprehensive recording of monitoring events, procedures and fates of individuals.

3.5.1. Introduction

Direct human interference was identified as one of the potential factors influencing the decline of woylies in the Upper Warren region. Direct human interference covers a wide range of activities and factors including; trapping intensity, live harvest for translocation, trapping consequences (deaths, predations and pouch young intervention), illegal killing/harvesting, and road kills.

This report focuses on trapping activities – in particular trapping intensity, pouch young intervention and live harvest events. These activities are viewed as being the most significant human factors which may affect populations, and ones which can be readily monitored and managed.

Ecotourism at Dryandra was found not to be compromising the welfare of woylies during a short-term study (Harvey 1999) Ecotourism in the Upper Warren is not as intense as that at Dryandra and is not investigated here. No data exists on illegal killing or harvesting of woylies, however it is not considered that this is an issue in the Upper Warren region.

Road kills are not considered to be a likely major contributing factor in the decline of woylies as many of the roads within the Upper Warren region are not open to the public and would receive very little use. There are no indications that traffic volume or behaviour has changed sufficiently over the past decade that could relate to the recent woylie declines. Where increased road kills have occurred in the past, it is considered to be an indication of increased population abundances rather than a contributing factor in their decline.

Foreign diseases could be introduced by humans into native fauna populations through release of animals from care, translocations, poor hygiene practices during monitoring or contact with domestic animals or introduced feral fauna. The impacts of disease and results of disease screening have been addressed in the disease section (Chapter 5).

The eleven Upper Warren Fauna Monitoring transects have been used to assess the potential impacts of direct human interference on woylie population declines, due to the existence of reasonable pre- and post-decline data for analysis.

3.5.2. Methods

Monitoring data and statistics from the eleven Upper Warren Fauna Monitoring transects (Chapter 2 UW Fauna Monitoring) were analysed to assess the potential impacts of human interference caused by monitoring and translocation activities.

To assess the potential impact of trapping intensity on woylie population declines, the number of trap nights per year was plotted against woylie capture rates (%TS) for each of the Upper Warren Fauna Monitoring transects. All cage trapping undertaken within the surrounding forest block has been included for the trap intensity – measured as total number of trap nights per year. The capture rates used relate to the Upper Warren Fauna Monitoring transects only (i.e. does not include other trapping that has occurred in the same forest block). In the case of three of the transects (Warrup, Winnejup and Keninup) capture rates have also been derived from historical monitoring transects which form sub-sets or super-sets of the Upper Warren Fauna Monitoring transects, in order to provide a greater case history for analysis.

Live harvest for translocation has occurred in the vicinity of only four of the Upper Warren Fauna Monitoring transects – Boyicup, Chariup, Camelar and Yackelup. The total number of woylie individuals live-harvested from a forest block has been compared with the capture rates of woylies on nearby Upper Warren Fauna Monitoring transects.

Joey intervention rates were analysed on only seven of the Upper Warren Fauna Monitoring transects due to the availability of suitably comprehensive data and/or the longevity of data.

3.5.3. Results

3.5.3.1. Trapping intensity

Six of the transects provide no substantial evidence for any relationship between the overall trap intensity within the forest block and capture rates of woylies (Figures 3.5.1, 3.5.3-4, 3.5.6-7 and 3.5.10).

Three of the forest blocks show a trend that the lowest woylie capture rates occur when trap intensity is generally highest on the transect (Figures 3.5.5, 3.5.8 and 3.5.9).

Increasing capture rates are associated with highest trap intensities on Warrup and Winnejup transects (Figures 3.5.2 and 3.5.11).

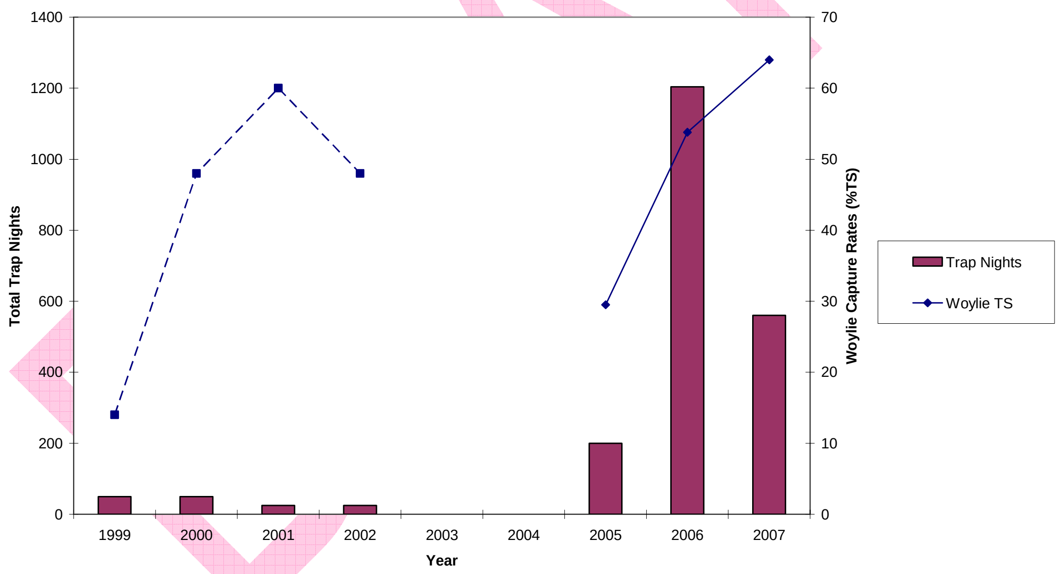


Figure 3.5.1. Relationship between trapping intensity and woylie capture rates on Keninup monitoring transect.

Note: The points joined by dashed lines indicate data derived from Keninup1 transect monitoring and those joined by solid lines indicate data derived from Keninup2 transect monitoring.

The data presented for 2007 represents the Mar/Apr trapping session only and does not include the Oct/Nov 2007 session.

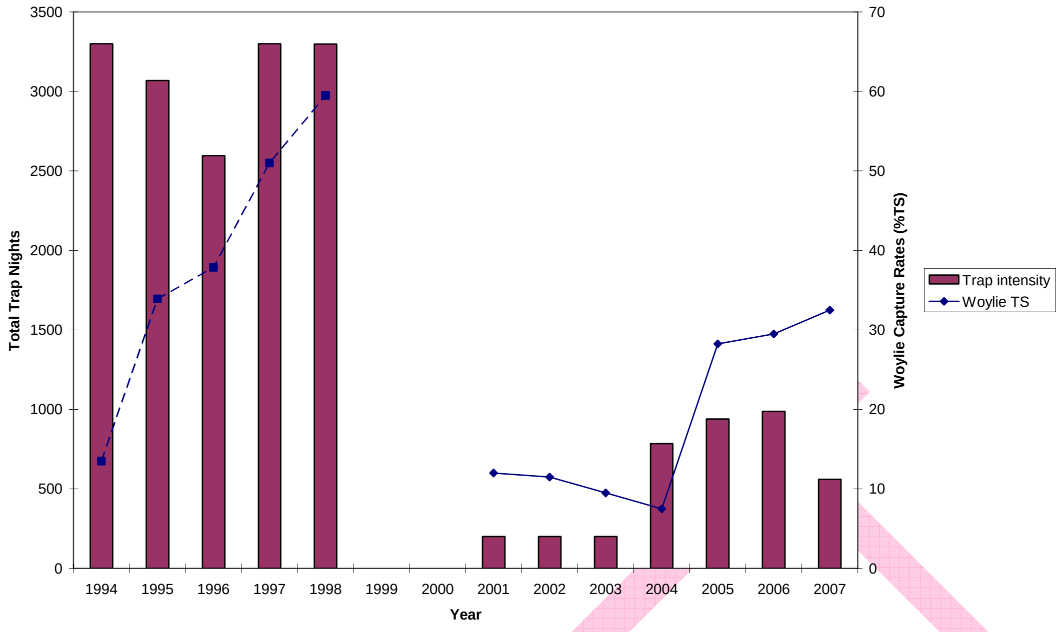


Figure 3.5.2. Relationship between trapping intensity and woylie capture rates on Warrup monitoring transect.

Note: The points joined by dashed lines indicate data derived from Warrup1 transect monitoring and those joined by solid lines indicate data derived from Warrup2 transect monitoring.

The data presented for 2007 represents the Mar/Apr trapping session only and does not include the Oct/Nov 2007 session.

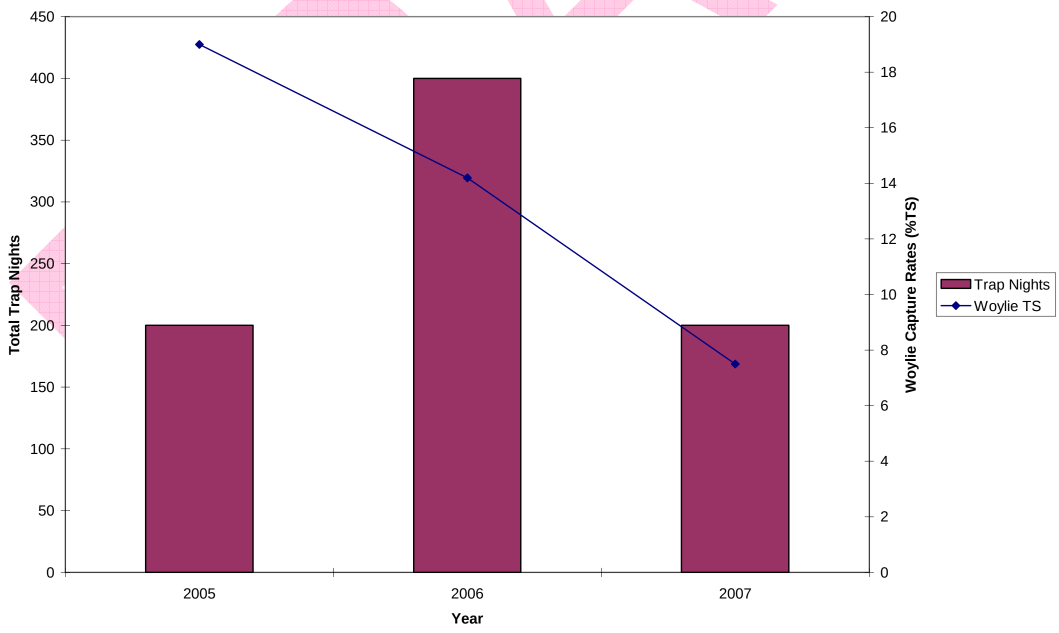


Figure 3.5.3. Relationship between trapping intensity and woylie capture rates on Corbal monitoring transect.

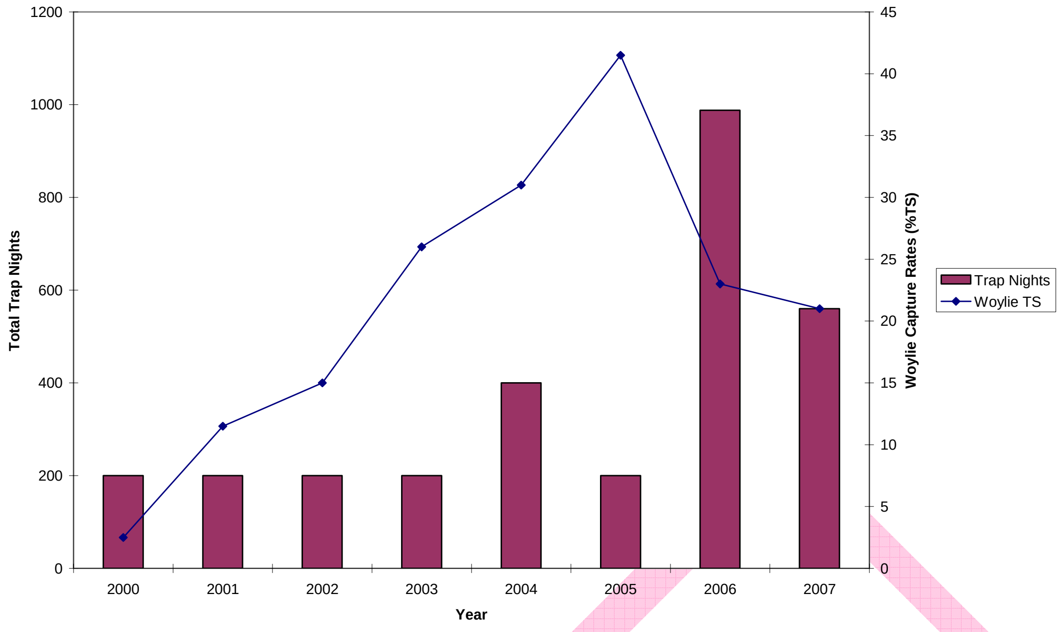


Figure 3.5.4. Relationship between trapping intensity and woylie capture rates on Balban monitoring transect.

Note: The data presented for 2007 represents the Mar/Apr trapping session only and does not include the Oct/Nov 2007 session.

Figure 3.5.5. Relationship between trapping intensity and woylie capture rates on Moopinup monitoring transect.

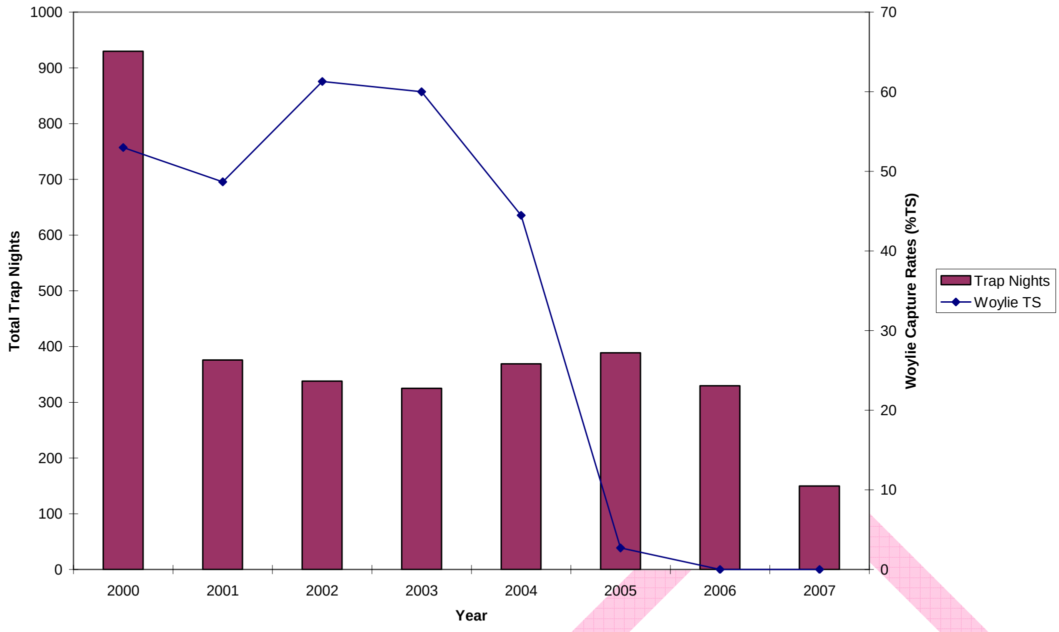


Figure 3.5.6. Relationship between trapping intensity and woylie capture rates on Yackelup monitoring transect.

Note: The data presented for 2007 represents the Mar/Apr trapping session only and does not include the Oct/Nov 2007 session.

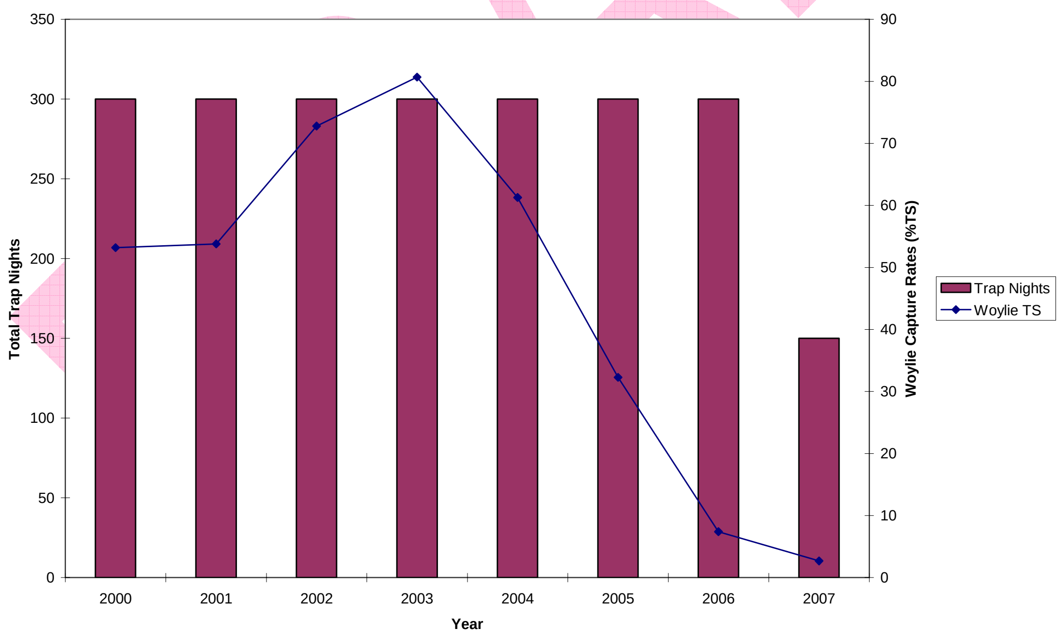


Figure 3.5.7. Relationship between trapping intensity and woylie capture rates on Yendicup monitoring transect.

Note: The data presented for 2007 represents the Mar/Apr trapping session only and does not include the Oct/Nov 2007 session.

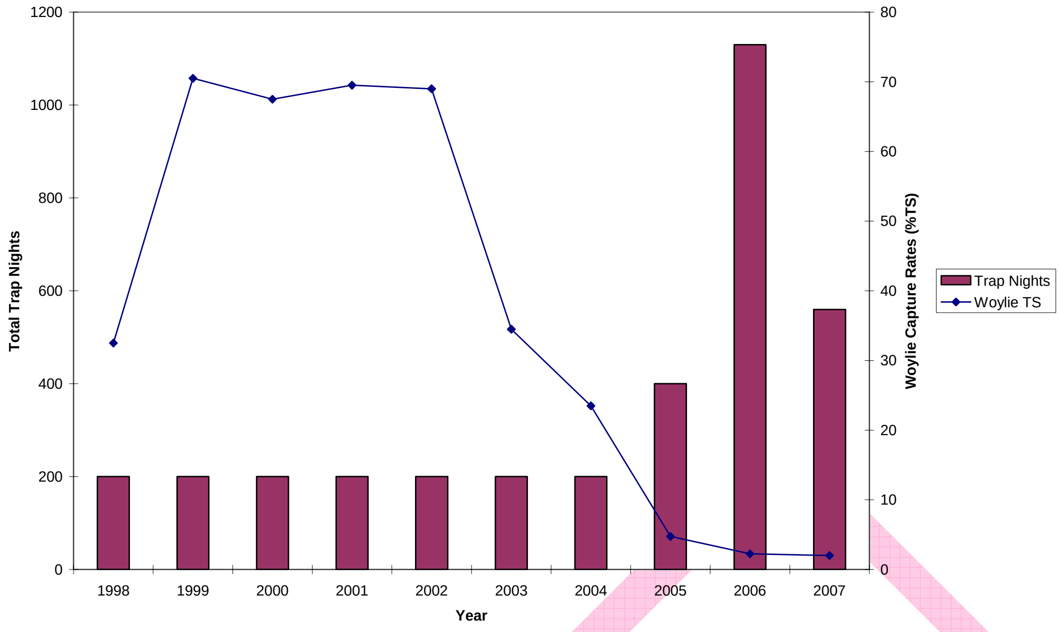


Figure 3.5.8. Relationship between trapping intensity and woylie capture rates on Boyicup monitoring transect.

Figure 3.5.9. Relationship between trapping intensity and woylie capture rates on Chariup monitoring transect.

Note: The dashed lines are indicative trends during the intervening periods between trapping events in non-successive years

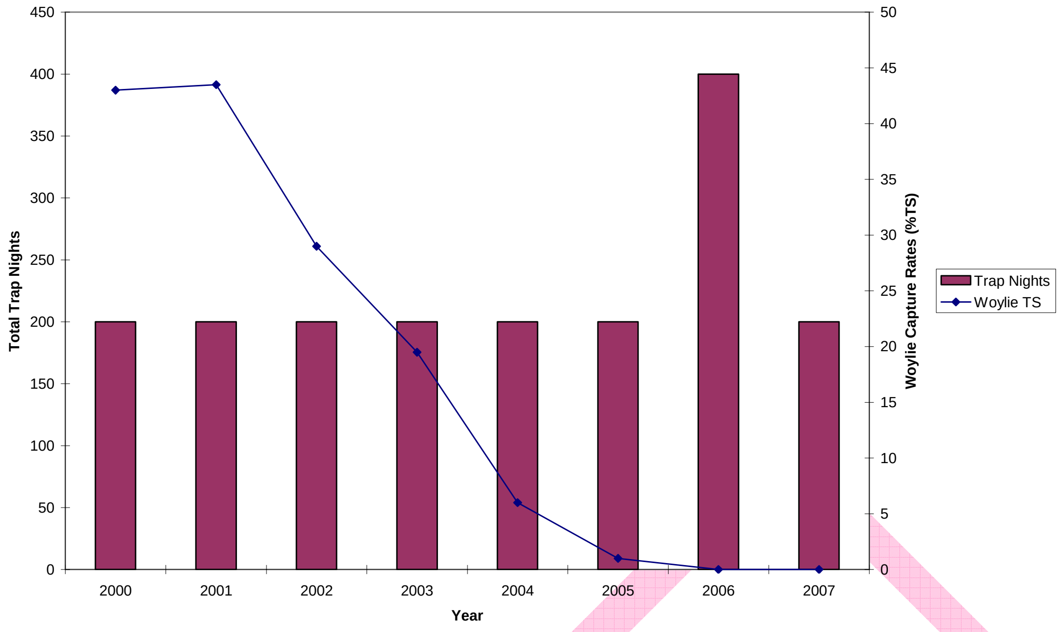


Figure 3.5.10. Relationship between trapping intensity and woylie capture rates on Camelar monitoring transect.

Note: The data presented for 2007 represents the Mar/Apr trapping session only and does not include the Oct/Nov 2007 session.

Figure 3.5.11. Relationship between trapping intensity and woylie capture rates on Winnejup monitoring transect.

3.5.3.2. Live harvest for translocation

Live-harvest of woylies for translocation occurred within Yackelup, Chariup and Boyicup forest blocks within the Upper Warren region. In 1998, 41 woylies were live-harvested from Chariup forest block, and 46 from Boyicup forest block. In 2000, 40 woylies were live-harvested from

Yackelup forest block. In 2002, 117 woylies were live-harvested from Yackelup forest block, and 35 from Chariup forest block.

Four transects (Yackelup, Camelar, Boyicup and Chariup) are located 2-5 km from the harvest sites. The woylie capture rates began to decline on these transects in 2004, 2002, 2003 and 2004 respectively.

No apparent relationships exist between; the number of individuals involved in, or timing of, live-harvest events and the commencement of woylie decline on nearby transects.

3.5.3.3. Other trapping-related impacts

Joey Intervention

The percentage of recorded woylie pouch young undergoing some form of human intervention (taping, bagging, ejections) varied considerably between years and transects, with no obvious patterns emerging (Table 3.5.1). The overall average annual joey intervention rate was 31.6 % (range – 20.6 to 47.1 %).

Table 3.5.1. Percentage and sample size (n) of recorded woylie pouch young with human intervention on seven of the Upper Warren Fauna Monitoring transects by year and averaged.

Year	Moopinup	Chariup	Boyicup2	Warrup2	Balban	Camelar	Keninup
1998	18.2 (11)	0 (13)	14.3 (21)	-	-	-	-
1999	3.8 (26)	10.3 (29)	12 (50)	-	-	-	-
2000	23.5 (17)	0 (9)	40 (5)	-	66.7 (3)	41.2 (17)	-
2001	71.4 (7)	64.3 (14)	77.8 (9)	-	11.1 (9)	22.2 (18)	-
2002	12.5 (8)	-	40 (15)	20 (5)	14.3 (14)	28.6 (7)	-
2003	57.1 (7)	26.7 (15)	14.3 (14)	66.7 (3)	18.8 (16)	33.3 (3)	-
2004	25 (8)	-	20 (5)	100 (2)	40 (15)	0 (2)	-
2005	100 (1)	15.8 (19)	75 (4)	33.3 (27)	16.7 (24)	-	12.5 (16)
2006	-	20 (5)	0 (2)	31.3 (32)	57.1 (14)	-	26.4 (72)
2007	100 (3)	27.3 (11)	0 (1)	31 (42)	31.3 (16)	-	24.7 (89)
Average	**45.7 (88)**	**20.6 (115)**	**29.3 (126)**	**47.1 (111)**	**32 (111)**	**25.1 (47)**	**21.2(177)**

3.5.4. Discussion

3.5.4.1. Trapping intensity

Varying associations can be identified between the trap intensity and capture rates of woylies in each of the 11 Upper Warren forest blocks examined (Figures 3.5.1-11).

Three of the forest blocks show lower woylie capture rates associated with higher trap intensities (Figures 3.5.5, 3.5.8-9). In each case the decline in woylie capture rates commenced two to three years prior to any increase in trapping intensity within the forest block (2005). In 2005 the possible decline in woylie populations was first noted which resulted in a response by Science Division and Donnelly District to conduct additional monitoring to substantiate and quantify the extent of the declines. After the declines were substantiated in 2005, the Woylie Conservation Research Project (WCRP) was established in 2006, involving increased monitoring across 11 key transects in the Upper Warren region (The Upper Warren Fauna Monitoring transects). The increased trapping intensity during this period is therefore a result of the response to the declines in woylie populations rather than a contributing cause of them.

Prior to 2005, there appears to be no relationship between the woylie capture rates and the trapping intensities within any of the 11 blocks examined. In most cases, the trapping intensity has remained relatively constant since the establishment of the monitoring transect, whilst capture rates have changed considerably over this period (Figures 3.5.1-11).

Of the eleven forest blocks, two have not undergone significant declines since 2004 despite the increased trapping intensity (Figures 3.5.1-2), and five of the forest blocks have undergone decline in woylie capture rates despite having had no significant change to the trapping intensity in recent years (Figures 3.5.5-8 and 3.5.10).

The combination of all of these factors mentioned above indicate that, throughout the Upper Warren region, there is no relationship between the trapping intensity and the decline in capture rates of woylies.

It should also be noted that the Karakamia woylie population experiences similar trap intensities, whilst maintaining very high population density, and displaying no signs of a decline (Section 4.2 Demographics). Similarly, live harvests and joey interventions (see below) have taken place at Karakamia without any apparent significant impact on the population (J Richards pers. comm.).

3.5.4.2. Live-harvest for translocation

A total of 279 woylies have been live-harvested over a seven year period (1998-2004) from an estimated pre-decline (2001) Upper Warren population of about 20,000 individuals (Adrian Wayne, pers. comm.). The live-harvested animals came from three localised areas within high density populations and constitute about 1% of the total estimated population. On this basis alone the live-harvest events can not be considered as a significant contributing factor in the rapid, substantial and widespread woylie declines in the Upper Warren region.

It is important to note that the woylie translocation harvesting sites were intentionally located away from long-term monitoring sites. Similarly, only un-tagged individuals were live-harvested for translocation, again, to minimize the impact on monitoring and research within the region. Therefore, the woylie capture rates for the Upper Warren Fauna Monitoring transects can not be used to directly monitor the impacts of live-harvest on the local populations. Nevertheless, there is no temporal relationship between the live-harvesting events and timing of the declines along the four Upper Warren Fauna Monitoring transects (Yackelup, Camelar, Boyicup and Chariup) located within moderate proximity (2-5 km) to the live-harvest sites. Furthermore, the declines on these four transects are characteristically similar to the declines that have occurred on other transects located significant distances from the live-harvest sites.

Therefore, given the extent and pattern of the declines in woylie populations within the Upper Warren region, the limited extent and locations of the woylie live-harvests and the lack of evidence of associated population change on the nearby transects; there is no evidence to suggest that live-harvest of woylies has been a significant contributing factor in the recent decline in woylie populations.

Nonetheless, harvesting of animals for translocations has the potential for significant but localized impacts on populations if not managed appropriately. Local management practices for wild translocations aim to ensure that live-harvesting is sustainable. To ensure this, monitoring of both the translocated and source populations should be undertaken to monitor success and impacts of translocations.

3.5.4.3. Other trapping related impacts

Trapping intensity directly influences the amount of exposure of woylies to human related impacts (such as joey pouch ejections, ‘tapings’, ‘baggings’ and risk of predation through day-time release). The lack of evidence for trapping intensity affecting woylie numbers would suggest a similar lack of evidence for these other potential human impacts on woylies.

Joey Intervention

There is no obvious pattern in the average pouch young intervention rates, relative to the extent of woylie population decline, across transects (Table 3.5.1). Camelar, which has one of the lower average intervention rates, has undergone a 100% decline in capture rates of woylies since 2001 (Figure 3.5.10). Warrup2, which has the highest average intervention rate, remains at moderate densities of woylies (Figure 3.5.2). It should also be noted that the average intervention rate for Keninup2 transect, where woylie numbers are high and decline has yet to commence, is 21.2%, which is within the range of the other transects analysed. From this it would appear that there is little evidence to suggest that a relationship exists between joey intervention rates and woylie population declines.

Joey intervention rates were high, with an overall average of 31.6% of all recorded joeys having some form of significant human intervention (ejections leading to tapings and baggings). This has the potential to have significant impacts on populations if these interventions are unsuccessful, particularly if trapping frequency and/or intensity is high.

It is therefore recommended that more comprehensive documentation of the results of interventions and fates of individuals be completed during monitoring sessions to enable review of current intervention procedures and monitoring protocols.

Woylie reproduction will be analysed further in Chapter 4 Population Comparison Study and Section 4.2 Demographics.

3.5.5. Future work

All future fauna monitoring, surveys and translocations involving capture of woylies will continue to observe strict adherence to the monitoring protocols set out in the 'Department of Conservation and Land Management Animal Ethics Standard Operating Procedures' (CALM, 2005) and the 'WCRP Operations Handbook' (Volume 3), as well as hygiene standards and protocols described in the 'Minimising Disease Risk in Wildlife Management' (Chapman *et al.*, 2005). This will ensure that potential human impacts are managed and minimised where possible.

Future monitoring will also involve comprehensive recording of the fates of all individuals captured, including pouch young (both those which have been directly intervened with through handling, taping and bagging; and those which have been passively affected through the capture and handling of the mother. Improved recording and monitoring will lead to improved handling and management techniques and protocols being developed, which can subsequently be incorporated into improved corporate standards. In the first instance, these points will be raised with the *Western Shield* Operations and Research Committee and the DEC Animal Ethics Committee.

It is important to ensure that monitoring efforts that aim to provide valuable information to assist in the conservation of threatened fauna species, do not in the process, detrimentally affect those species.

3.5.6. Conclusion

Given that human activities may impact on woylie populations it is important to monitor these potential impacts through adequate recording of monitoring events, procedures and fates of individuals. Likewise, it is important that adequate monitoring of both translocated and source populations be undertaken to monitor success and impacts of translocations.

Despite the potential for significant impact, there is no evidence in the Upper Warren region that trapping activities or live-harvesting for translocations have been a major contributing factor in the decline of woylie populations.

3.5.7. References

CALM, 2005. Department of Conservation and Land Management Animal Ethics Standard Operating Procedures. Department of Conservation and Land Management, Perth.

Chapman, T., Sims, C., Mawson, P., 2005. Minimising Disease Risk in Wildlife Management. Standard operating procedures for fauna translocation, monitoring and euthanasia in the field. Department of Conservation and Land Management, Perth.

Harvey, N. 1999. The impact of ecotourism upon the wellbeing of woylies at Dryandra Woodland village. Honours Thesis. University of Western Australia.

Wayne, A.F., Wilson, I., Northin, J., Barton, B., Gillard, J., Morris, K., Orell, P., and Richarson, J., 2006. Situation report and project proposal: identifying the cause(s) for the recent declines of woylies in southwestern Australia. A report to the Department of Conservation and Land Management Corporate Executive. CALM, Perth, Western Australia.

3.6. Fire

Julia Wayne, Adrian Wayne and Ian Wilson

Department of Environment and Conservation.

Abstract

A number of fire history attributes were analysed in relation to woylie capture rates and extent of decline along the 11 Upper Warren Fauna Monitoring transects.

Corporate fire history data and District and Science Division fauna monitoring data were used in the analyses.

No relationships were found during these or previous analyses of fire history data and woylie capture rates and declines.

These are preliminary analyses only, and more sophisticated and rigorous analysis of the data is required to verify whether any temporal patterns or relationships exist.

3.6.1. Introduction

It is possible that fire may be a potential contributing factor in the recent rapid and substantial declines in woylie populations in the south-west.

Attributes of fire history which may impact on fauna populations include fire frequency and interval, fire intensity, fire size, time since last fire, proximity to fire edges and diversity of times since last fire (fire age) (e.g. Friend and Wayne, 2003).

Impacts of fire on fauna populations may include direct loss of individuals, interruptions to breeding cycles, loss and regeneration of habitat (shelter and vulnerability to predation), and disruption and change to food resources.

Fire history can be very complicated as multiple variables need to be considered. 'Fire units' describe the patches or areas of similar recent fire history that result from the management of fire in discrete units or areas, which at the landscape-regional scale result in a mosaic of different fire histories. Fire units can change over time and the fauna monitoring transects (~10 km long) typically intersect several different fire units. Over the last 50 years up to eight or more fire events may have taken place at each fire unit and therefore the combination of fire history variables can be numerous.

A previous analysis of fire history data was conducted by Adrian Wayne for the DEC workshop on 'Recent mammal declines' held at Perup on 16th and 17th February 2006. No significant relationships were found between fire history attributes and woylie abundances (measured by capture rates) during this earlier analysis (Wayne, 2006). (Volume 2 Appendix 1) This analysis provides an update using current woylie capture rates and decline figures. It should be noted that these are preliminary analyses, which look at a single point in time. More rigorous and sophisticated analyses, which look at fire history from a broader temporal perspective, are required to more completely address these issues.

3.6.2. Methods

Data from the 11 Upper Warren Fauna Monitoring transects (Chapter 2 UW Fauna Monitoring) were used in this investigation. Woylie capture rate data from March/April 2007 were used in these analyses. The percentage decline in woylie capture rates for each transect is derived from the woylie capture rate (%TS) in April 2007 divided by the average woylie capture rate immediately prior to decline along the same transect.

Attributes of fire history were analysed in relation to woylie capture rates for each of the 11 Upper Warren Fauna Monitoring transects. Fire history attributes were analysed in relation to percentage decline for only a sub-set of the Upper Warren Fauna Monitoring transects. This was due to limited or incomplete trapping histories for some transects (Keninup2, Corbal, Winnejup and Warrup2) that made determination of pre-decline woylie capture rates (and therefore percentage decline) and/or the year of commencement of decline impossible.

The attributes of fire which were analysed included:-

- The number of 'fire ages' (years since last burn) occurring along the length of the transect (fire age diversity)
- The proximity of the transect to the adjacent fire unit boundaries ('boundary' or 'core') – relative to the majority of the transect (proximity to fire units).
- The earliest year of last burn along the transect (oldest fire age)
- The latest year of last burn along the transect (youngest fire age)
- The predominant season of the most recent fire events adjacent to the transect (coarse season last burnt)
- The average number of times the area adjacent to the transect has burnt between 1953 and 2005 (fire frequency).

Corporate fire history data from 2005 (McCaw *et al.,* 2005) was used in the development of the dataset used for these analyses. This data was prepared by Adrian Wayne for an earlier analysis of the relationships between woylies and fire, presented at the Mammal declines workshop at Perup in February 2006. Given that there were minimal differences in the fire history between February 2006 and the time of this analysis, this pre-existing dataset was considered the most efficient means of further exploring the relationships between fire and woylies. Since 2005, fire history data has changed on two of the Upper Warren Fauna Monitoring transects (Moopinup and Warrup2). Moopinup transect is within the core of a burn conducted in spring 2006; however this burn was extremely mild and patchy with less than 30% of the area burnt. The majority (>90%) of the area immediately adjacent to the monitoring transect has remained unburnt. Due to the limited amount of the transect being affected by this burn and the timing of the burn being well after commencement of decline in woylies, this fire event has not been included in the analysis. Similarly, a burn was conducted adjacent to a section (approximately 15%) of the Warrup2 transect in spring 2006. The burn was conducted on one side of the road only. This fire event affects the 'youngest fire age' attribute only, and due to the limited amount of the transect being directly affected, this burn event has not been included in the analysis.

Statistical analyses principally involved regression analyses and single factor ANOVA tests.

3.6.3. Results

3.6.3.1. Summary of fire history

A summary of the data for the variables used in these analyses are provided in Table 3.6.1.

Table 3.6.1. Summary of woylie population characteristics and fire history for each of the 11 Upper Warren Fauna Monitoring transects.

TRANSECT	WOYLIE CAPTURE RATES APRIL 2007	DECLINE TO APRIL 2007	PROXIMITY TO FIRE UNITS	FIRE AGE DIVERSITY	OLDEST FIRE AGE	YOUNGEST FIRE AGE	SUMMARY OF SEASON LB	COARSE SEASON LB	FIRE FREQUENCY 1953-2005
Balban	21.0	69%	Core	1	2003	2003	Su	Au	5.4
Boyicup2	2.0	97%	Core	3	1985	1997	Au/Sp	Au	5.4
Camelar	0.0	100%	Boundary	2	2003	2004	Au/Sp	Au	7.2
Chariup	5.0	92%	Core	1	1997	1997	Sp	Sp	4.4
Corbal	8.0	~	Boundary	4	1992	2002	Sp	Sp	6.7
Keninup2	64.0	~	Core	3	1995	2003	Au	Au	5.3
Moopinup	3.0	95%	Core	2	1988	1995	Au	Au	6.2
Warrup2	33.0	33%	Boundary	6	1975	2003	Sp/Au	Sp	6.8
Winnejup	5.0	85%	Boundary	3	1985	2002	Au/Sp	Au	7.9
Yackelup	0.0	100%	Core	1	1999	1999	Au	Au	6.1
Yendicup2	3.0	95%	Core	2	1954	1968	Sp	Sp	3.3

Seven of the 11 Upper Warren Fauna Monitoring transects were situated predominantly within the core of fire units, with three of these situated entirely within one fire unit (i.e. only one fire age along the entire transect). The remaining four transects were located predominantly along the boundary of fire units with up to six different fire ages adjacent to a transect.

The year of last burn varied greatly between transects, and also within transects with greater fire age diversity. The average number of fire events (fire frequency) occurring adjacent to each transect over the 52 year period from 1953 to 2005 ranged from 3.3 to 7.9.

3.6.3.2. Fire history attributes in relation to woylie capture rates

No relationships were found between the capture rates of woylies and the fire history attributes of fire age diversity, fire frequency, oldest or youngest fire age (Figures 3.6.1-4).

No significant relationships were found between the extent or commencement of decline in woylie capture rates and the youngest fire age for those transects analysed in the Upper Warren region (Figure 3.6.5 and 3.6.9 respectively). Similarly, no relationships were evident between the extent of woylie capture rate decline and fire age diversity, oldest fire age or fire frequency (Figures 3.6.6-8).

No relationships were evident between either woylie capture rates or the extent of decline and either the proximity to fire units or the coarse season last burnt (p-value = >0.2 in all cases) (Table 3.6.2-5).

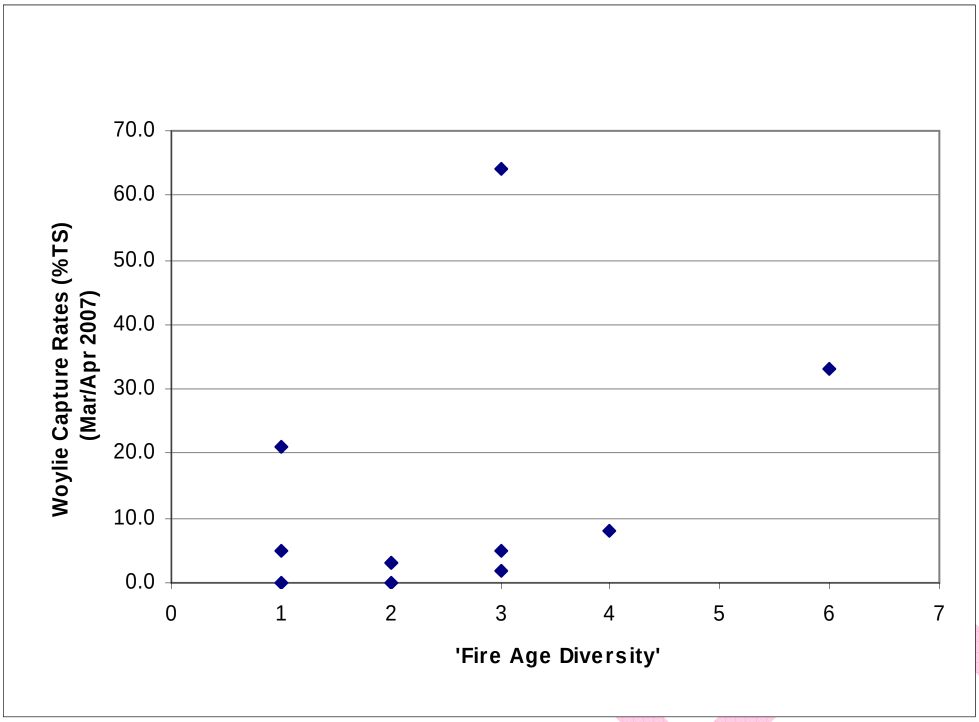


Figure 3.6.1. Relationship between fire age diversity and woylie capture rates along each of the Upper Warren Fauna Monitoring transects.

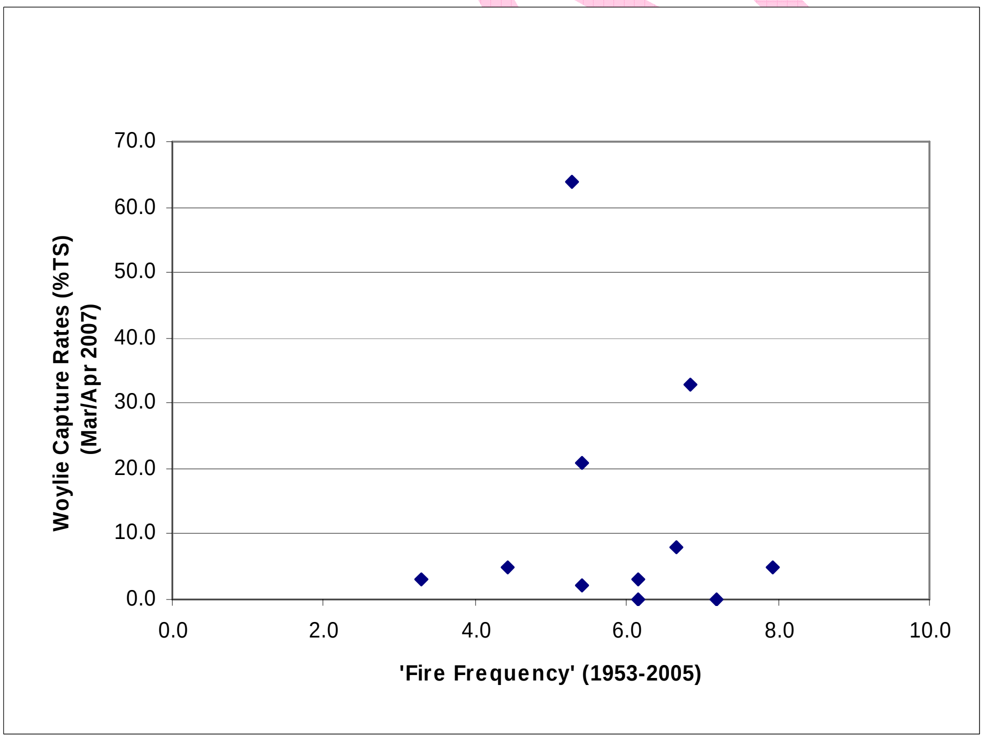


Figure 3.6.2. Relationship between fire frequency (average number of fire events from 1953-2005) and woylie capture rates along each of the Upper Warren Fauna Monitoring transects.

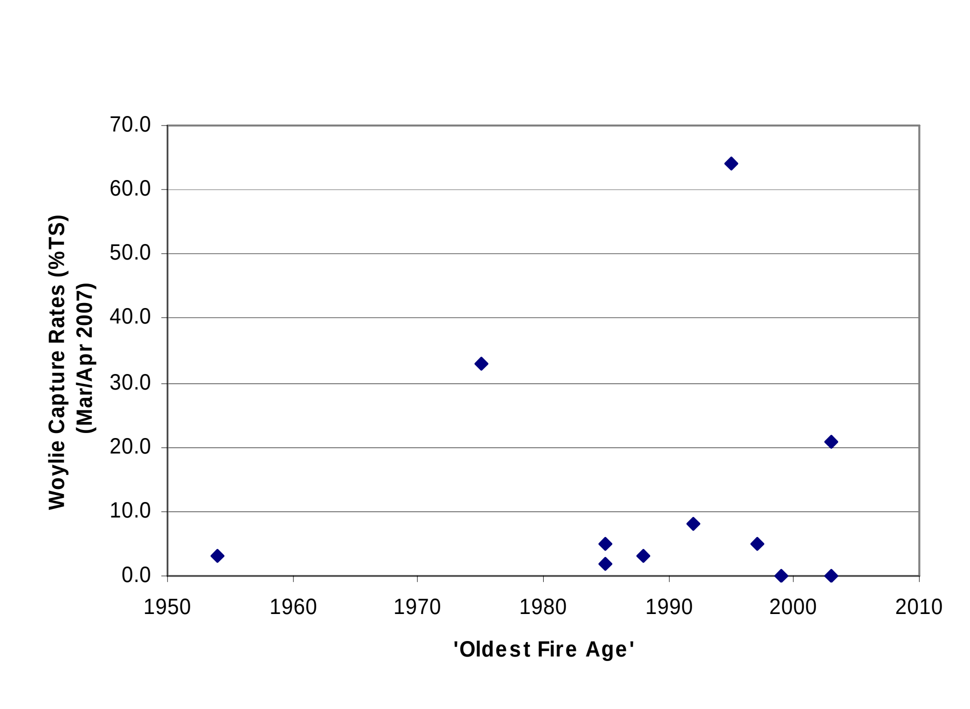


Figure 3.6.3. Relationship between oldest fire age (earliest year last burnt) and woylie capture rates along each of the Upper Warren Fauna Monitoring transects.

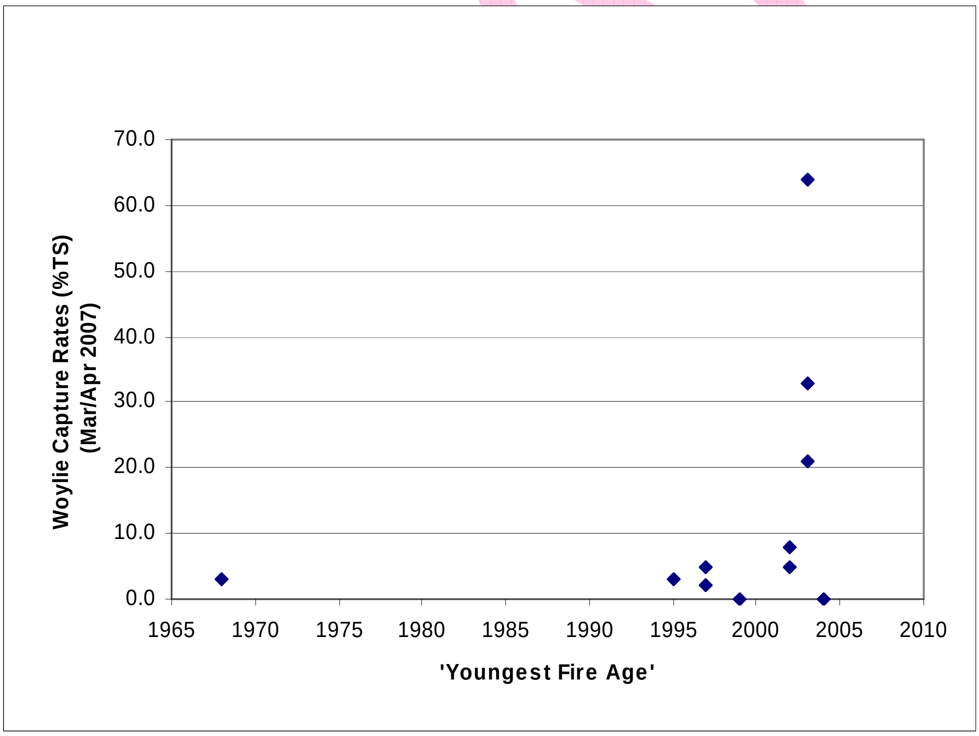


Figure 3.6.4. Relationship between youngest fire age (latest year last burnt) and woylie capture rates along each of the Upper Warren Fauna Monitoring transects.

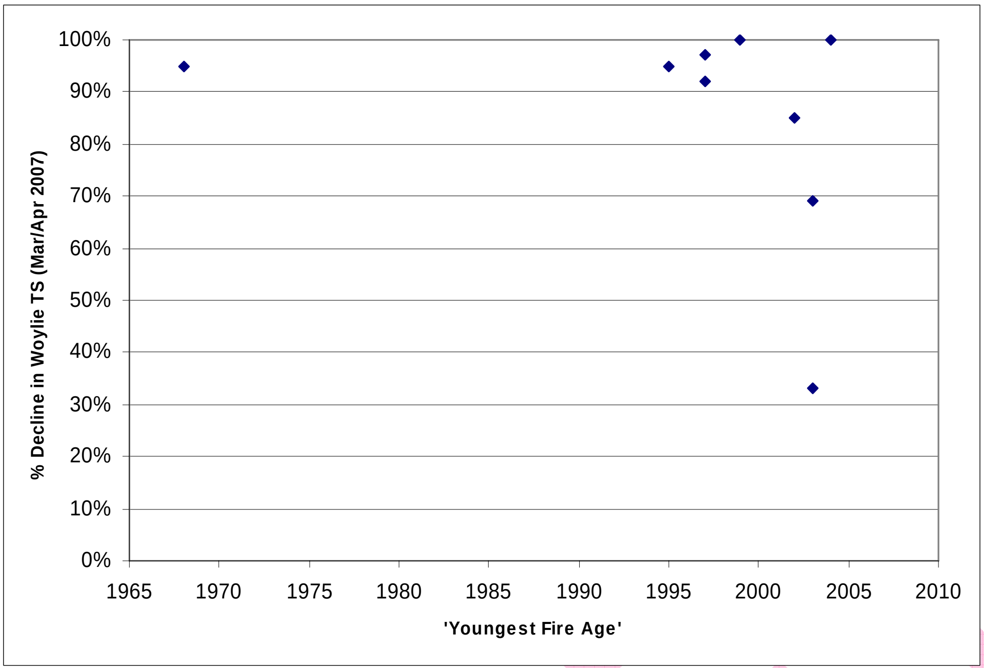


Figure 3.6.5. Relationship between youngest fire age and extent of decline in capture rates along nine of the Upper Warren Fauna Monitoring transects.

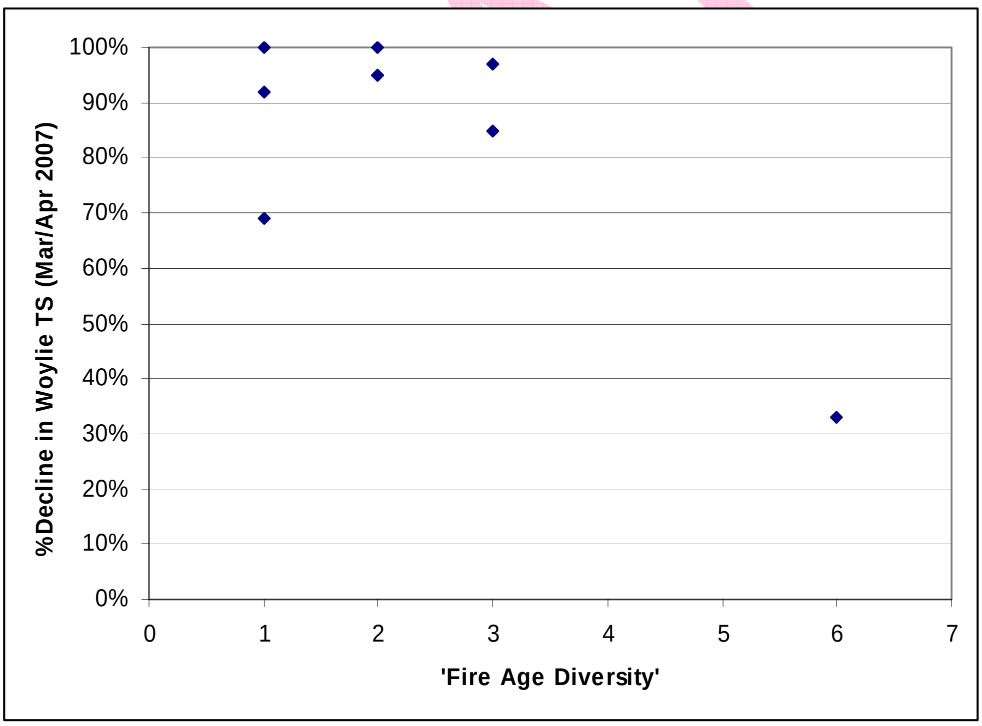


Figure 3.6.6. Relationship between fire age diversity (number of fire ages along the transect) and extent of decline in capture rates along nine of the Upper Warren Fauna Monitoring transects.

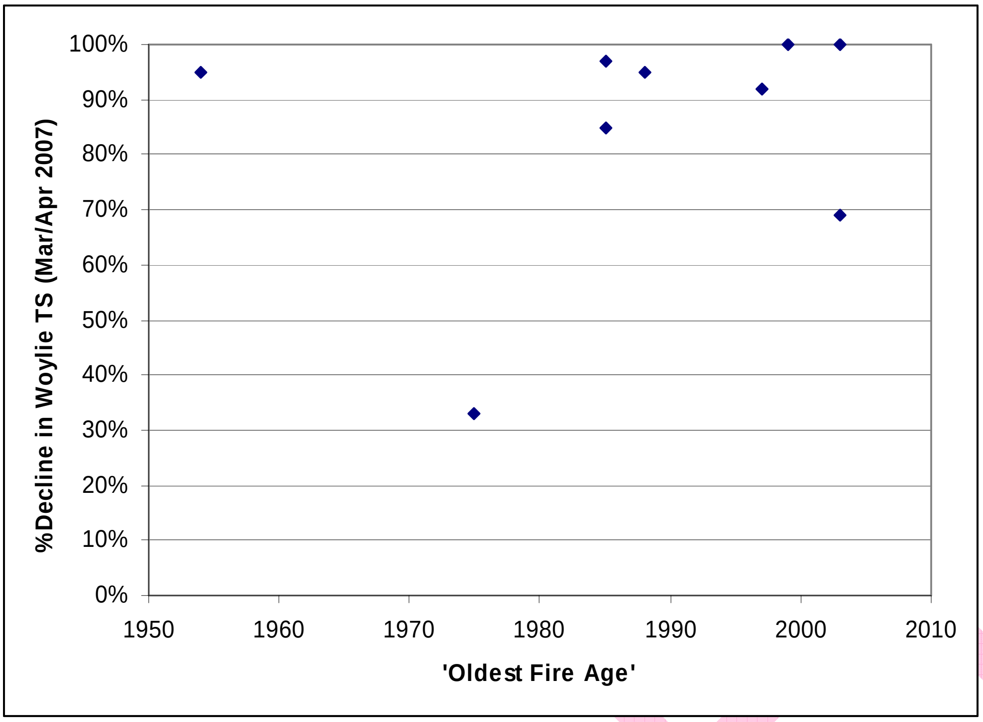


Figure 3.6.7. Relationship between oldest fire age and extent of decline in capture rates along nine of the Upper Warren Fauna Monitoring transects.

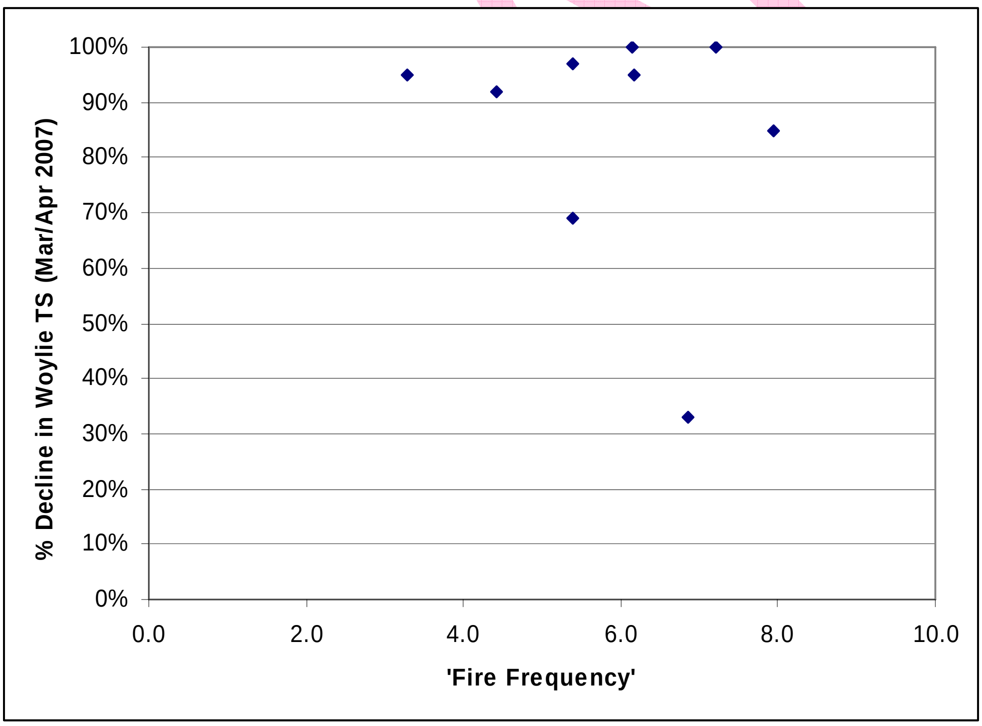


Figure 3.6.8. Relationship between fire frequency (average number of fire events between 1953 and 2005 along the transect) and extent of decline in capture rates along nine of the Upper Warren Fauna Monitoring transects.

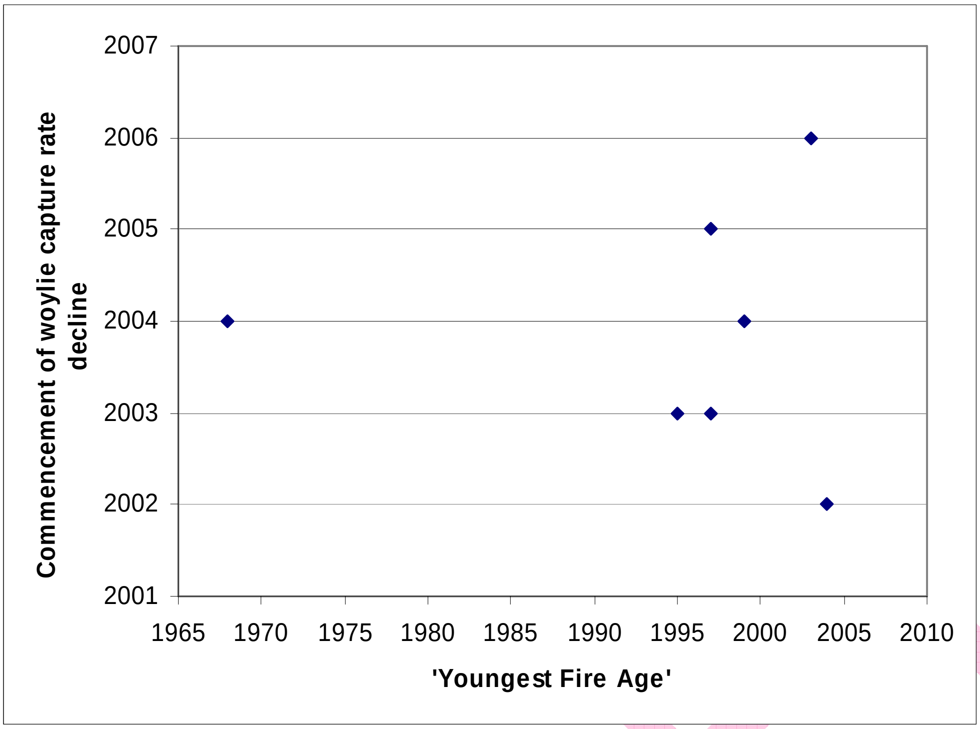


Figure 3.6.9. Relationship between youngest fire age and commencement of woylie capture rate decline along seven of the Upper Warren Fauna Monitoring transects.

Table 3.6.2. Relationship between proximity to fire units and woylie capture rates along nine of the Upper Warren Fauna Monitoring transects.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Core	7	98	14	535.3333
Boundary	4	46	11.5	216.3333

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	15.90909	1	15.90909	0.037084	0.851569	5.117355
Within Groups	3861	9	429			
Total	3876.909	10				

Table 3.6.3. Relationship between proximity to fire units and extent of decline in capture rates along nine of the Upper Warren Fauna Monitoring transects.

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Core	6	548	91.33333	126.6667
Boundary	3	218	72.66667	1236.333

ANOVA

Source of	SS	df	MS	F	P-value	F crit
Between Groups	696.8889	1	696.8889	1.57058	0.25036	5.591448
Within Groups	3106	7	443.7143			
Total	3802.889	8				

Table 3.6.4. Relationship between coarse season last burnt and woylie capture rates along nine of the Upper Warren Fauna Monitoring transects.

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Au	7	95	13.57143	547.619
Sp	4	49	12.25	195.5833

ANOVA

Source of	SS	df	MS	F	P-value	F crit
Between Groups	4.444805	1	4.444805	0.01033	0.921273	5.117355
Within Groups	3872.464	9	430.2738			
Total	3876.909	10				

Table 3.6.5. Relationship between coarse season last burnt and extent of decline in woylie capture rates along nine of the Upper Warren Fauna Monitoring transects.

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Au	6	546	91	146.8
Sp	3	220	73.33333	1222.333

ANOVA

Source of	SS	df	MS	F	P-value	F crit
Between Groups	624.2222	1	624.2222	1.37465	0.279366	5.591448
Within Groups	3178.667	7	454.0952			
Total	3802.889	8				

3.6.4. Discussion

The preliminary analyses indicate that no relationships were evident between various attributes of fire history and woylie capture rates or contemporary extent of decline.

Of particular note, youngest fire age (i.e. latest year of last burn) adjacent to the transect showed no relationship to woylie declines. The 'Yendicup2' transect, which last burnt in 1968, has undergone similar extent of decline as several other transects with considerably younger fire ages (Figure 3.6.5). Similarly, there were no trends evident between the timing of the commencement of woylie declines and the most recent fire event associated with the fauna monitoring transects (Figure 3.6.9).

The limited number of data points used to investigate the potential for fire history relationships with woylie decline is a substantial constraint of these preliminary analyses. More sophisticated analyses that can incorporate a much broader temporal context to the possible relationships are needed to more robustly address these questions. Having said this, there is no evidence yet that indicates that fire is involved in any way with the recent rapid and substantial woylie declines and it remains unlikely that such evidence might exist. For example, there is nothing remarkable about the nature and pattern of fire that has changed substantially between the 1990's and early 21st century that could account for the woylie declines in the Upper Warren. This includes factors such as fire interval, burn size, burn season rotation, and local- and landscape-scale fire heterogeneity, that have not altered substantially over the past decade or so (Rod Simmonds, pers. comm.).

Previous research suggests that there may be immediate and acute responses to fire by woylies, followed by rapid recovery within four to five years (Christensen, 1980). However, other research has found little or no evidence of this. For example Burrows and Christensen (2002) found 'no discernable impact of fire on capture rates of native mammals (including the woylie), with trends in capture rates being independent of the time since the last fire'. This also supports the findings of this analysis. Friend and Wayne (2003) provide further frameworks which may aid in understanding or predicting woylie responses to fire.

Fauna responses in other studies have often been more closely related to vegetation changes than to specific attributes of fire, such as fire age (Catling *et al.*, 2001; Fox, 1996; Monamy and Fox, 2000). On this basis it might be more instructive to investigate woylie responses to vegetation structure and floristics, to complement these direct investigations into fire. These relationships should be addressed as part of the ongoing work regarding the population comparison study of woylie resources. (Chapter 4 Population Comparison Study, Section 4.5 Resources)

Similar analyses of this fire history data were conducted by Adrian Wayne in 2006, with the same lack of relationships being observed.

3.6.5. Future work

Further analysis is required to determine whether there are any relationships between fire and recent woylie declines. Due to the multiple variables involved, this will require more sophisticated and rigorous analyses that incorporate the broader temporal context within these potential relationships. An analysis of the unpublished woylie data from the Batalling study (Friend and Wayne, 2003) that specifically examined the fauna responses to prescribed burning would also be directly relevant to these investigations.

3.6.6. Conclusion

The preliminary investigation described here, found that no relationships were evident between woylie capture rates or extent of decline and various fire history attributes, including fire age diversity, year of last burn (oldest and youngest fire age), coarse burn season, proximity to fire units and fire frequency. These results are consistent with an earlier examination of the same factors conducted in February 2006. Furthermore there is no evidence (either contemporary or published), or ecological basis that indicates that fire may have a substantial role in the recent rapid and substantial woylie declines observed in the Upper Warren, Dryandra, Batalling or elsewhere. More sophisticated and rigorous analyses are required to verify these preliminary findings.

3.6.7. Acknowledgements

We would like to thank Tom Hamilton and Lachie McCaw for the access to the Corporate fire history data set and their support in its use for this investigation.

3.6.8. References

Burrows, N. D., and P. E. S. Christensen. 2002. Long-term trends in native mammal capture rates in a jarrah forest in south-western Australia. Australian Forestry **65**:211-219.

Catling, P. C., N. C. Coops, and R. J. Burt. 2001. The distribution and abundance of ground-dwelling mammals in relation to time since wildfire and vegetation structure in south-eastern Australia. Wildlife Research **28**:555-564.

Christensen, P. E. S. 1980. The biology of *Bettongia penicillata* Gray 1837, and *Macropus eugenii* Desmarest 1817, in relation to fire. Bulletin 91. Western Australian Government Forests Department, Perth.

Fox, B. J. 1996. Long-term studies on small mammal communities from disturbed habitats in eastern Australia. Pages 467-501 in M. L. Cody, and J. A. Smallwood, editors. Long-term studies of Vertebrate Communities.

Friend, G. R., and A. F. Wayne. 2003. Relationships between mammals and fire in south-west Western Australian ecosystems: what we know and what we need to know. Pages 363-380 in I. Abbott, and N. D. Burrows, editors. Fire in Ecosystems of South-West Western Australia: Impacts and Management. Backhuys Publishers, Leiden, The Netherlands.

McCaw, L., T. Hamilton, and C. Rumley. 2005. Application of fire history records to contemporary management issues in south-west Australian forests in M. C. Calver, H. Bigler-Cole, G. Bolton, J. Dargavel, A. Gaynor, P. Horwitz, J. Mills, and G. Wardell-Johnson, editors. 6th National Conference of the Australian Forest History Society inc. Millpress Science Publishers, Rotterdam.

Monamy, V., and B. J. Fox. 2000. Small mammal succession is determined by vegetation density rather than time elapsed since disturbance. Austral Ecology **25**:580-587.

Wayne, A. F. 2006. Fire and woylie abundance. In Presentation and proceedings of the 'Recent mammal declines in the southwest workshop' Perup, 16 February 2006.

3.7. Climate

Peter Orell

Department of Environment and Conservation

Abstract

Rainfall data from Bureau of Meteorology recording stations located near *Western Shield* fauna monitoring sites in southwestern Australia were plotted as annual, monthly, winter and summer rainfall with line plots for long-term averages as a reference. Visual comparison of the data with capture rates of woylies and other medium size mammals at fauna monitoring sites suggests that there may be a weak link with decline in rainfall and population decline in the lower rainfall sites in the wheatbelt but the link is less apparent in the higher rainfall sites in the jarrah forest. The evidence suggests that climate is not a primary causative agent in the decline of woylies at least in the Upper Warren region.

3.7.1. Introduction

It is generally recognized that climatic factors such as rainfall can drive the population dynamics in some animal species such as granivorous rodents. The amount and timing of rainfall generally determines the amount of seed production of local plant species and therefore determines the amount of food resources available for these rodents. Other factors such as predation may also influence the population dynamics but in some systems rainfall seems to be the driving force and has a stronger influence than other climatic factors.

When population declines of woylies in Dryandra were first reported during the review of *Western Shield* in 2003 it was postulated that these declines might be associated with below average rainfall in recent years combined with other factors such as predation (Orell, 2004). Examination of rainfall data over the seven year period in which dramatic declines followed large population peaks seemed to support the hypothesis.

Anecdotal evidence from South Australia suggests that the declines in woylies observed on Venus Bay Peninsula (VBP) beginning in 2005 may be attributed to a food resource decline associated with postulated woylie over-population and drought conditions in combination with predation by feral cats. Extreme and uncharacteristic weather (a series of six severe frosts in a week) may also have contributed to the woylie declines at VBP (Section 4.6 PCS Expansion).

The experiences in both Western Australia and South Australia suggested that climate could be a likely factor in the observed declines and was worthy of closer examination.

3.7.2. Methods

Bureau of Meteorology (BOM) weather recording stations in the south west of WA were selected on the basis of their proximity to *Western Shield* fauna monitoring sites and the consistency of data recorded. Of the data available, rainfall appeared to be the most consistently recorded data for all the stations selected and was therefore chosen for further investigation.

Rainfall data from the following recording stations was sourced from BOM.

Pingelly (January 1990 – December 2005) as data for Tutanning Nature Reserve;

Cuballing (January 1990 – December 2005) as data for Dryandra Woodland;

Pingrup (January 1990 – December 2005) as data for Lake Magenta Nature Reserve;

Valern (January 1990 – December 2005) as data for Batalling Forest; and

Westbourne (January 1975 – December 2005) as data for Perup.

The data were plotted as: monthly rainfall together with a line plot of the monthly average since records were started; annual rainfall with a line plot of the annual average since records were started; winter (May to October) rainfall with a line plot of the average winter rainfall since records were started; and summer (November to April) rainfall with a line plot of the average summer rainfall since records were started.

These plots were examined for potential patterns that might be correlated with the observed declines at the respective fauna monitoring sites. No statistical analyses were undertaken.

3.7.3. Results

A visual examination of the plots of rainfall data in comparison with the fauna monitoring data for the respective sites suggests that the declines observed in Dryandra (Fig.3.7.1), Tutanning and Lake Magenta (Fig.3.7.2) could be partly explained by a decline in winter rainfall since 2000. Near average rainfall in 2003 and above average rainfall in 2005 was recorded in Pingrup (Fig.3.7.2) and this appears to correlate with an increase in koomal and chuditch captures from May 2004. A similar pattern in rainfall, but lower in relation to the average rainfall, was observed in Pingelly but a continued decline in capture rates of mammals occurred at Tutanning.

The rainfall data for Valern (Fig.3.7.3) and Westbourne (Fig.3.7.4) do not show any visible correlation with capture rates of woylies at either Batalling or Perup respectively. Increases in woylie captures occurred despite periodic declines in rainfall.

3.7.4. Discussion

While a cursory examination of rainfall data from recording stations in south west WA suggests that declines observed in wheatbelt populations might be partly associated with a decline in rainfall, the association is much weaker in the higher rainfall sites in the jarrah forest.

A spatial analysis of rainfall data and woylie trapping results from 1995 to 2002 by Criddle (2004) at the same sites as discussed here suggested there was no link between rainfall and woylie population decline, though the data available was insufficient to draw firm conclusions.

As suggested by anecdotal evidence from South Australia, a combination of factors, including other climatic variables like temperature, may be playing a role in the decline of woylie populations. A further investigation into variables like temperature, evapotranspiration and ground water levels may be worth while in gaining a better understanding of the environment in which these declines are occurring.

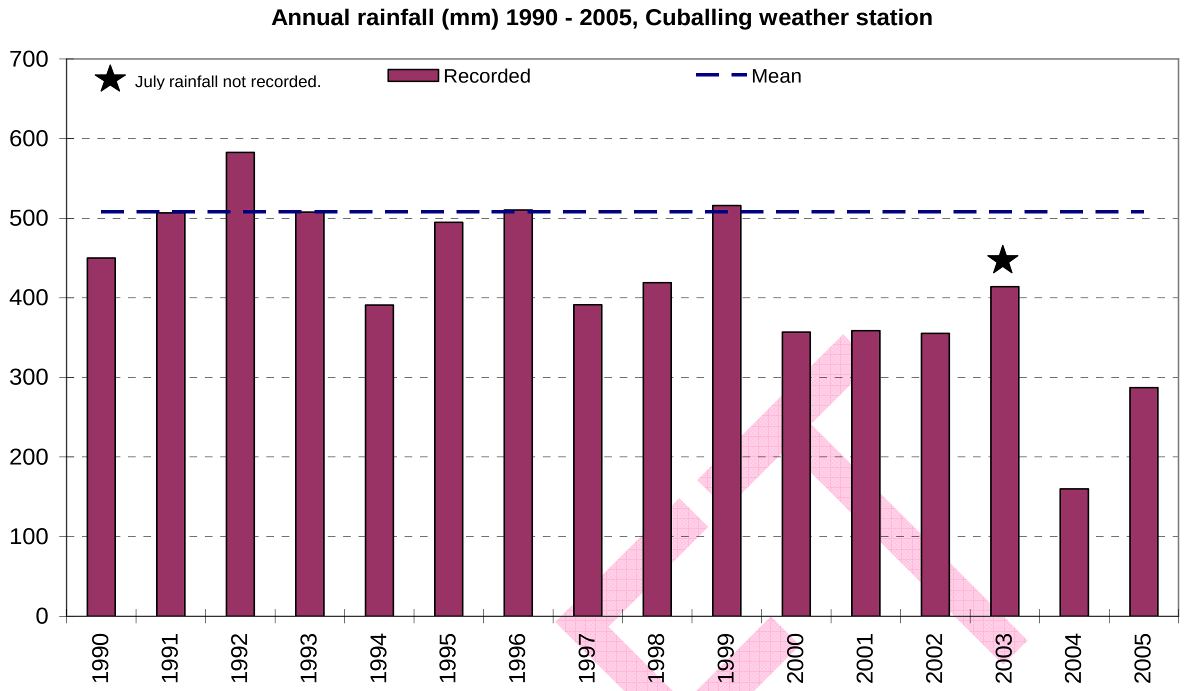
3.7.5. Conclusion

There appears to be a weak link between the observed declines in woylie populations in southwestern Australia and rainfall but this cannot be substantiated without more detailed data and statistical analysis. However, the evidence suggests that rainfall or climate is not a primary factor in the decline of woylie populations

3.7.6. References

Criddle, L. 2004. Analysis of woylie distribution through the south west of Western Australia. Student project report, Curtin University of Technology, Bentley, Western Australia.

Orell, P. 2004. Fauna monitoring and staff training: *Western Shield* review – February 2003. *Conservation Science W. Aust.* **5** (2): 51-95.



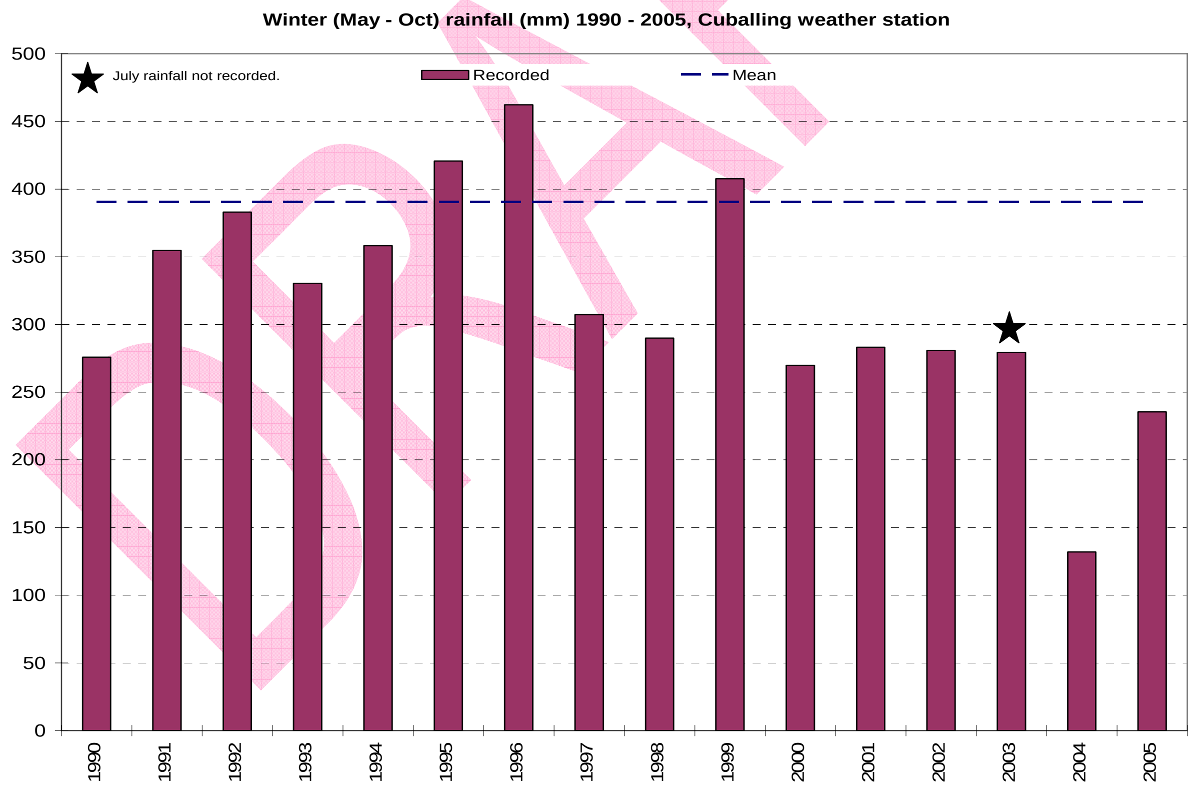
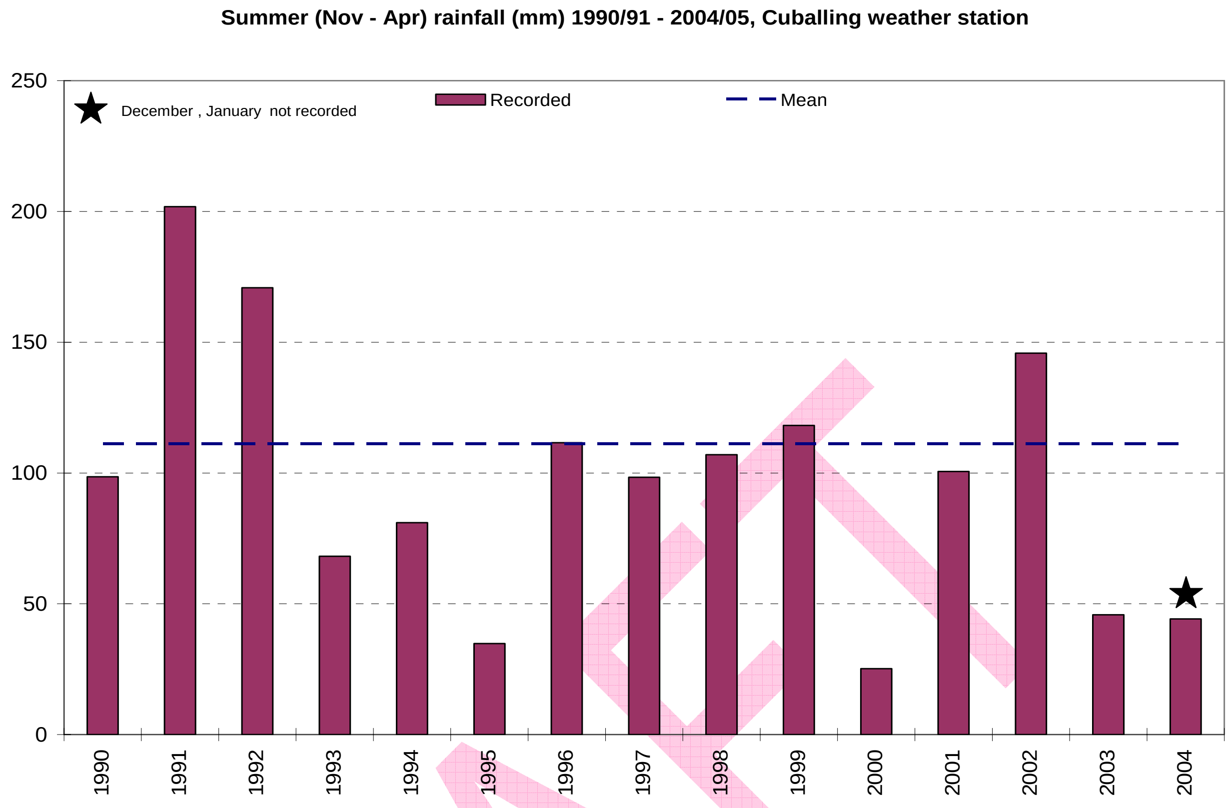


Figure 3.7.1a Cuballing (Dryandra) rainfall – total annual and winter rainfall.



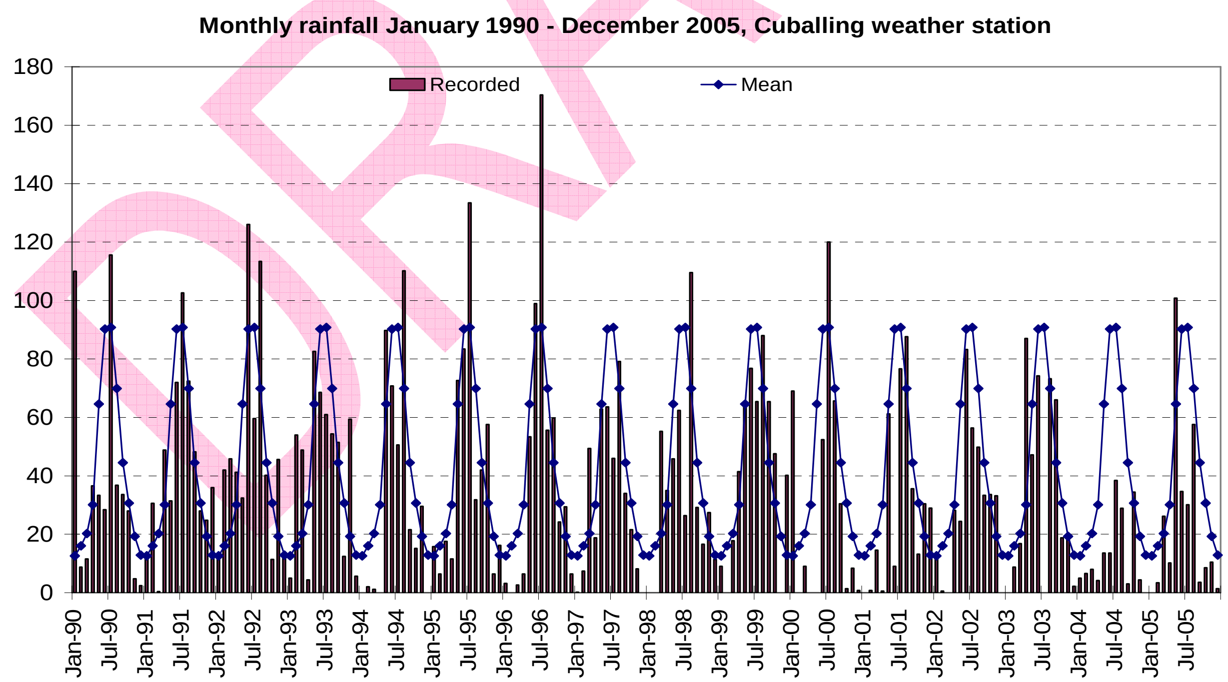
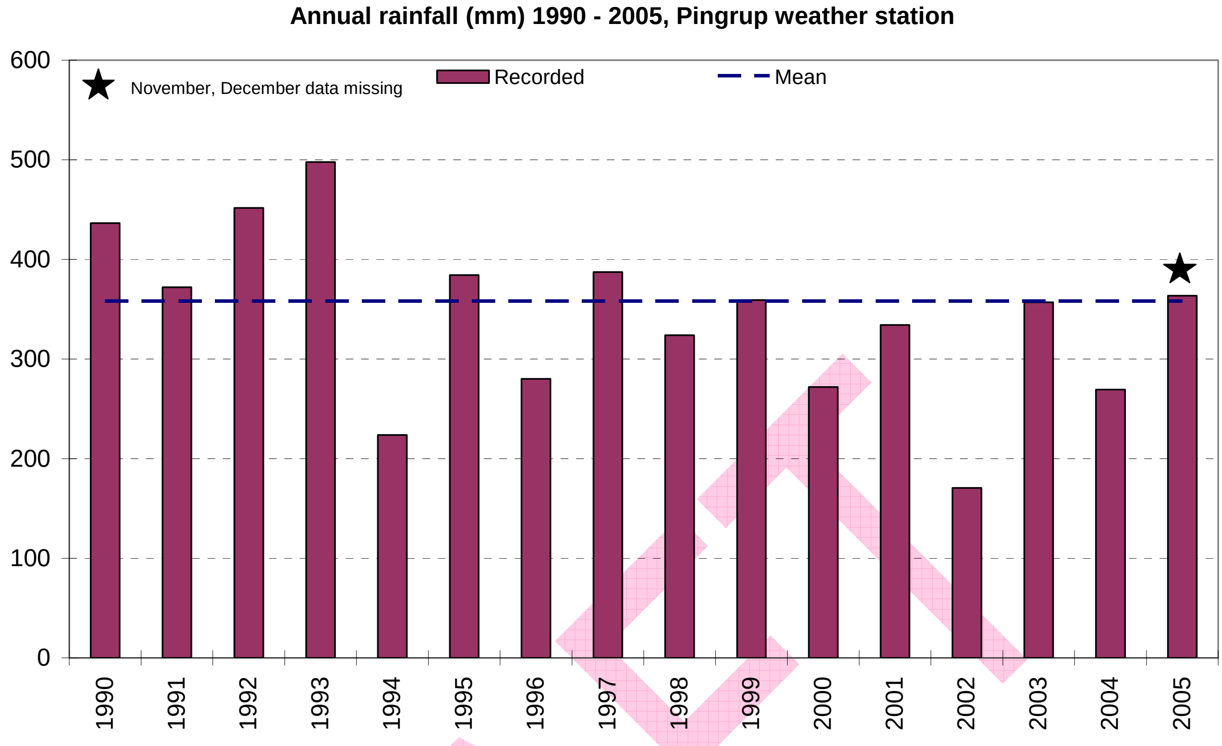


Figure 3.7.1b Cuballing (Dryandra) rainfall – total summer rainfall and monthly rainfall.



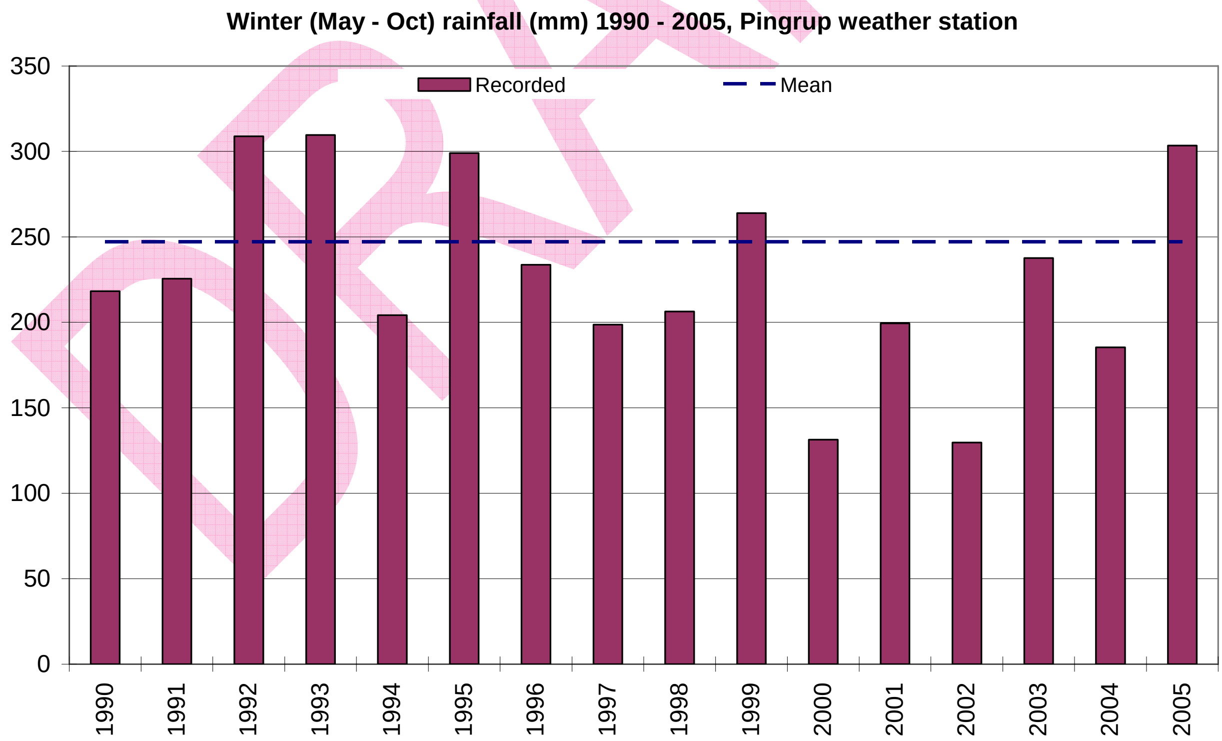
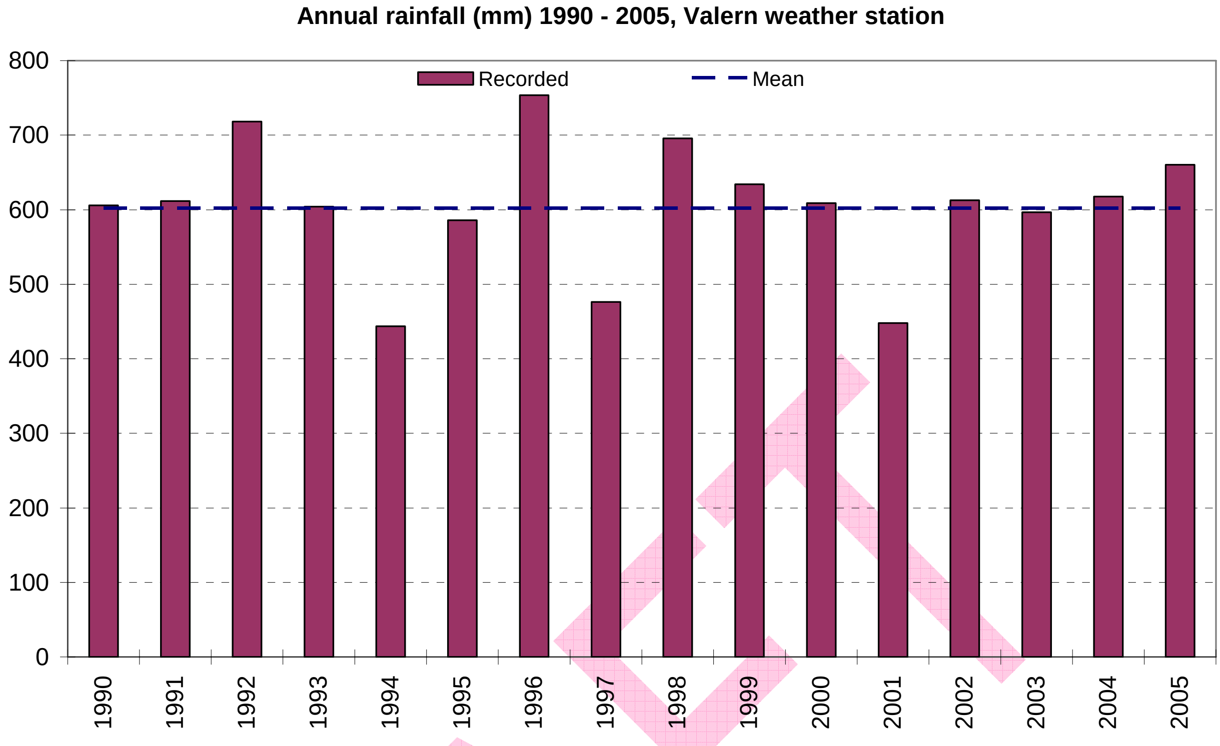


Figure 3.7.2. Pingrup (Lake Magenta) rainfall – total annual and winter rainfall.



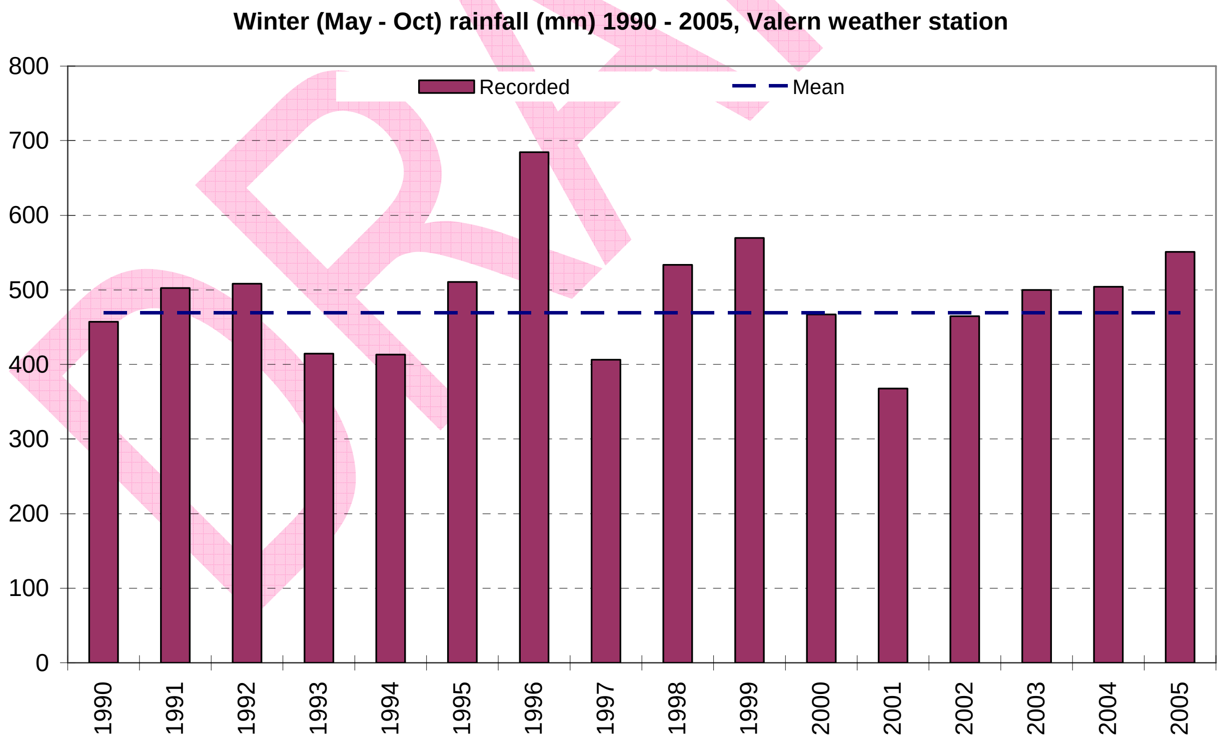
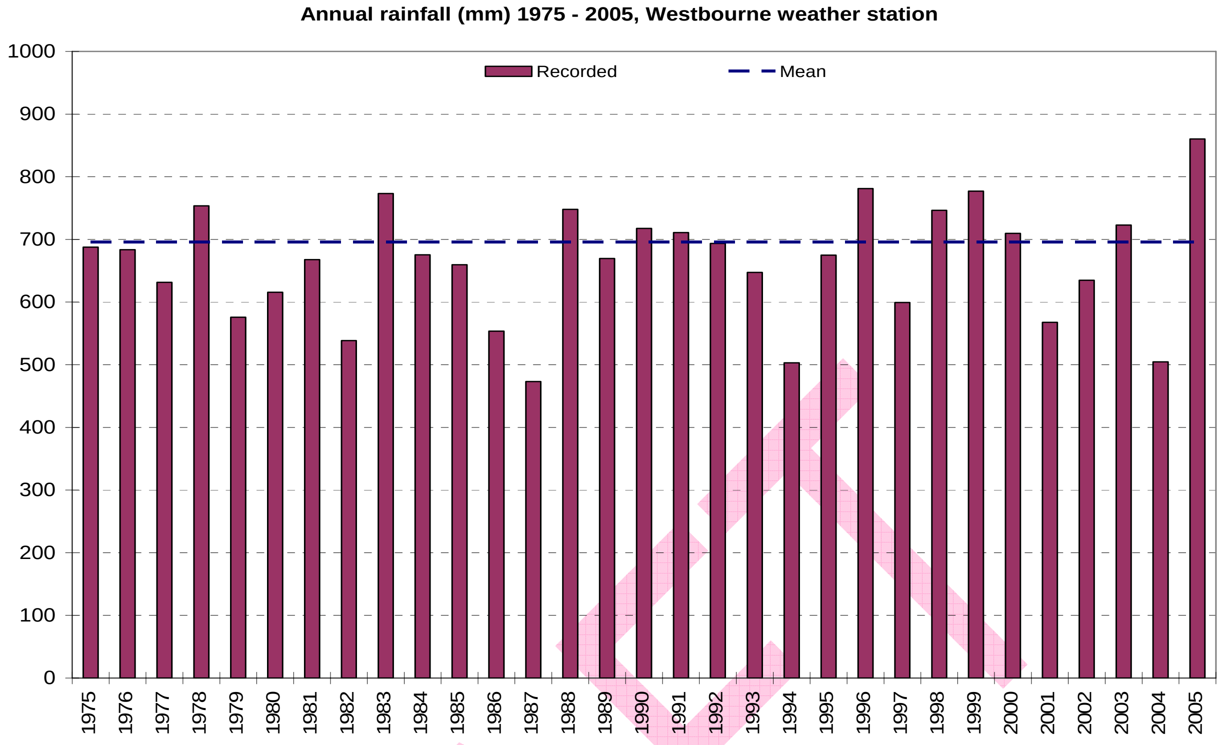


Figure 3.7.3. Batalling rainfall – total annual and winter rainfall.



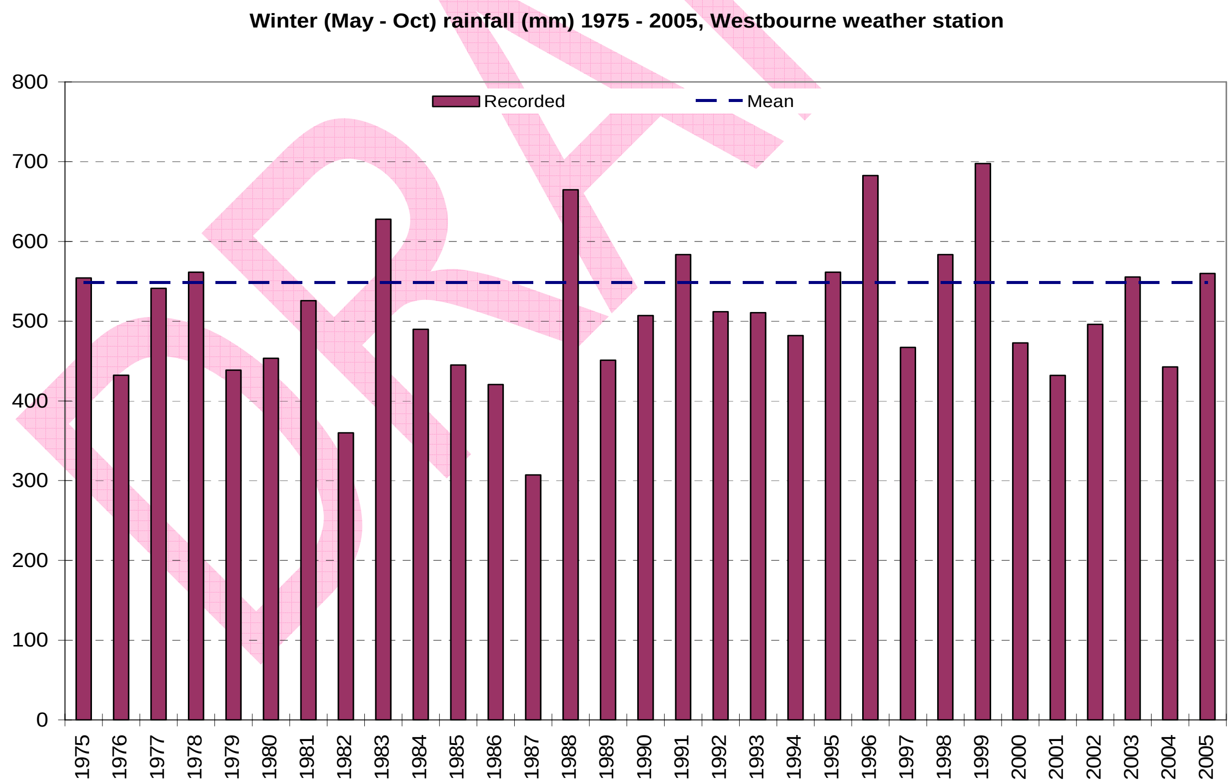


Figure 3.7.4. Perup rainfall – total annual and winter rainfall.

CHAPTER 4 POPULATION COMPARISON STUDY

4.1. Overview

Adrian Wayne

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4.1.1. Introduction

The quickest and surest means to identifying the agent(s) of decline is to use a hypothetico-deductive approach (Caughley, 1994, Caughley and Gunn, 1996). The declining-population paradigm provides a framework by which this approach can be used. Comparisons between populations at different stages of decline provide strong associative evidence that can identify putative agent(s) of decline among the countless conceivable possibilities. The 'shortlist' of putative agents that result from this exercise can then be experimentally tested to robustly determine their role in a species decline.

The Population Comparison Study (PCS) component of the Woylie Conservation Research Project (WCRP), is therefore, the principal tool in the diagnosis of woylie decline. The section provides a brief overview of the design and details of the PCS common to its various components.

4.1.2. PCS investigative components

The five investigative components of the PCS, examined differences between the woylie populations and the three major categories of putative agents of decline;

Woylie components

1. Woylie demographics
2. Woylie survival and mortality

Putative agents of decline

3. Predators
4. Resources
5. Disease

Together, these components provide an extensive and integrated enquiry into some of the mechanics possibly causing the woylie declines and the possible factors that may be associated.

4.1.3. PCS sites

This is a more detailed and focused study designed to complement data collected from the broad-scale regional (Upper Warren) fauna monitoring program (Chapter 2). This study focused on the Upper Warren region where moderate woylie densities still existed and where declines were current. The five PCS sites in the Upper Warren region were associated with a subset of the 11 key monitoring transects (Figure 4.1.1) and provided replicated representation of contemporary population states across the region;

- Declined populations now at low densities: Boyicup and Winnejup
- The last remnant moderate-density populations: Keninup, Warrup and Balban (i.e. sites seemingly not yet affected and which have the potential to decline)

Karakamia Wildlife Sanctuary supports the last remaining high density woylie population. Managed and owned by the Australian Wildlife Conservancy and isolated from other woylie populations, the woylies at Karakamia are contained within 275 ha of jarrah/marri forest, bounded by a predator-proof fence (i.e. cat and fox free). Having been reintroduced to Karakamia in 1996, the woylie numbers rapidly expanded and have remained at relatively stable, high densities for about the last five years (Trish Gardner, pers. comm.). Located approximately 50 km east of Perth, Karakamia provides a particularly powerful comparative site to the Upper Warren PCS sites.

4.1.3.1. Site descriptions

The five Upper Warren PCS sites were located adjacent to some of the key fauna monitoring transects. The PCS site at Karakamia was approximately central to the property. In contrast to the transects used for regional fauna monitoring, the PCS research was site-based, using the trapping grids associated with the demographics component as the central points of reference. The predator and resources components necessitated studying sites outside these grids but remained closely associated with the trapping grids wherever possible.

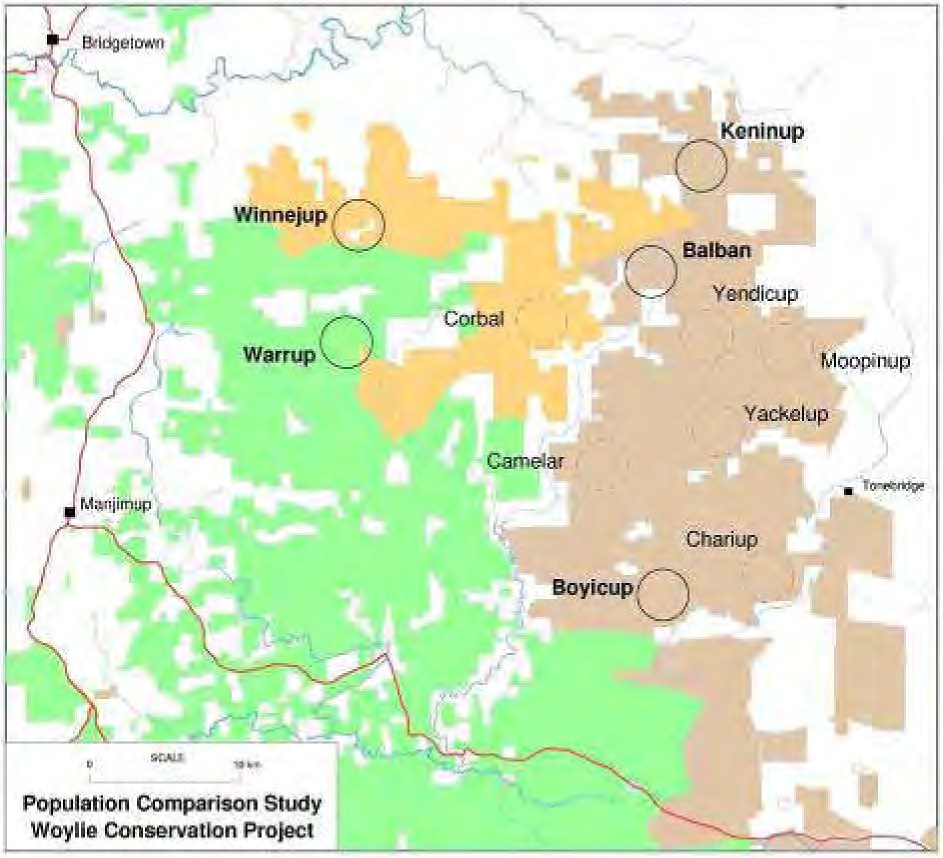

Figure 4.1.1. The five principal Upper Warren areas (in bold) associated with the Population Comparison Study in relation to the other sites associated with the Upper Warren Fauna Monitoring program (plain text and dashed circles). The Karakamia PCS site is 50 km east of Perth, Western Australia.

4.2. Demographics

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Abstract

The biometric and demographic attributes of woylie populations were examined as part of the Population Comparison Study (PCS). The five PCS grids in the Upper Warren and the one PCS grid at Karakamia Wildlife Sanctuary were live cage-trapped every eight weeks for 12 months (Upper Warren sites, July 2006 - June 2007; Karakamia, September 2006 – July 2007). The capture rates of woylies declined at Balban (21% to 4%), remained relatively stable and high at Karakamia (mean 77%), stable and moderate at Keninup (mean 52%) and Warrup (mean 29%), and remained low, having declined in the preceding years at Winnejup (mean 3%) and Boyicup (mean 2%).

Preliminary exploration of the data included the temporal and spatial variation in demographic attributes of the six populations. Sex ratios differed substantially between the moderate-density sites in the Upper Warren (strongly male biased) and Karakamia (strongly female biased). The prevalence of subadults was low or absent at all sites but varied temporally at Karakamia. The proportion of adult females breeding within the Upper Warren region was similar between grids and over time (overall mean of 89%) but highly seasonal at Karakamia (4% in summer to 81% in spring). Adult woylies at Karakamia were smaller than at Upper Warren sites (means of 1070 g and 1368 g, respectively). More thorough and extensive analyses of the demographic attributes of these woylie populations are planned.

4.2.1. Introduction

A comparative study of woylie populations at varying densities and stages of decline should provide evidence relating to what factors may and may not be associated with recent woylie population declines. For example, the mechanics by which a population may decline may vary substantially according to the factor(s) involved. Some factors may affect particular components of the population differently, such as individuals of different age, gender, breeding status, size or condition. Fecundity, life expectancy, and/or behaviour (e.g. emigration) may also change.

The results of a preliminary exploration of the trap capture rates and demographics of woylies at the six Woylie Conservation Research Project (WCRP), Population Comparison Study (PCS) grids are presented here. These initial findings are briefly discussed and the plans for further, more formal analyses are outlined.

4.2.2. Methods

Trapping grids were established at each of the five Upper Warren PCS sites (Keninup, Balban, Warrup, Boyicup, Winnejup; each adjacent to the associated monitoring transects) and at Karakamia Wildlife Sanctuary. The grids were trapped from July 2006 to June 2007 at the Upper Warren and from September 2006 to July 2007 at Karakamia. Trap sessions were repeated every eight weeks (i.e. approximately half the duration of the woylie breeding cycle) and were conducted simultaneously within Upper Warren region wherever possible (occasionally within the same fortnight). Each trap session was conducted over four nights an exception was the august session which was primarily conducted for applying radio transmitters here trapping ceased when radio collared targets were achieved.

Within each grid, trap points (marked by GPS-referenced metal fence droppers) were spaced 50 m apart in lines oriented to magnetic north-south and east-west. During the first trapping session at the Upper Warren sites, the grid consisted of 25 traps (5 lines of 5 traps each, i.e. 200 m x 200 m). In response to the low number of woylies captures on the low density sites, the grid was expanded to 49 traps (7 lines of 7 traps each, i.e. 300 m x 300 m) in the aim of getting >10 woylie individuals on each of the PCS grids. These initial trap sessions indicated that grids several orders of magnitude, and well beyond available resources, would be required to capture >10 woylie individuals per trap session on the low density sites. As a result the grids were subsequently reduced and maintained at 30 traps (5 lines of 6 traps each, i.e. 200 m x 250 m) owing to the resource and logistical constraints and ethical considerations of processing all animals prior to 1100 hrs, particularly on the more abundant sites.

The data collected from captured animals are provided in detail in the WCRP Operations Handbook (Volume 3) but included standard biometric measurements, detailed information on reproductive status and pouch-young development and detailed health checks (Section 5.2 Field health and disease sampling). These trapping sessions were also used to source woylies for radio-collaring as part of the survival and mortality component of the PCS study (Section 4.3). These and other selected animals were also sampled (blood, faeces, ectoparasites and ear tissue) for integrated disease (Chapter 5), resource (Section 4.5) and genetic (Chapter 6) studies.

Capture rates (i.e. trap success) are the proportion (%) of the number of animal captures relative to the number of traps available. Wayne (2006) demonstrated that capture rates were strongly related to more sophisticated population abundance measures, such as minimum number known to be alive (MNA or KTBA) and mark-recapture population models (e.g. POPAN), albeit a more conservative measure of population change. These alternative measures of population abundance will be considered for future analyses, however, during these preliminary explorations of the PCS data, capture rates were used for woylies, koomal and other species (including quenda, chuditch and varanids).

4.2.3. Results

4.2.3.1 Karakamia historical data summary

- Trap success has remained high since 1999, four years after the first translocation.
- Trapping sessions tended to be female-biased in the early days of population establishment (1994 – 1996) and then more commonly male-biased as the population size and density increased (1999 onwards). More recently female-biased sex ratios were recorded in trapping results between September 2006 and May 2007. When only individuals born at Karakamia were taken into consideration, the overall sex ratio of woylies was 453:402 males:females (1.13:1, 53% male), displaying a slighter greater male bias than represented with the captured population.
- Adult woylies were regarded as weighing ≥ 750 g as at this weight many females were carrying pouch young. Some females carried pouch young at a much lower body weight.
- 97% of woylies captured at Karakamia were adults, only 3% subadults < 750 g. It was not possible to draw any conclusions about capture of subadults due to the paucity of these captures.
- Periods of a significant reduction in breeding occurred in January/February 1995 and January 2007. Unfortunately trapping was not conducted in January or February during any other year since 1995 however observations during spotlight walks have suggested that woylies continued to breed over the summer months between 1996 and 2006.
- Body weight of adult woylies was lower at Karakamia than the Upper Warren sites and this occurred right from 1995 when the colony commenced.
- There is some indication that average body weight has declined over time at Karakamia, from around 1170 g in 1995 to 1070 g in 2007.
- All female captures combined over time (1090 g) were slightly heavier on average than males (1075 g).

4.2.3.2. Trapping summary

The number of trap nights remained relatively consistant after an initial period when trapping to apply collars was occurring (Table 4.2.1). The woylie captures varied spatially between PCS trapping grids (Table 4.2.2). The average number of woylie individuals caught on Keninup, Balban, Warrup Boyicup and Winnejup were 28, 8, 17, 2 and 2 respectively. Karakamia caught an average of 43 individuals (Table 4.2.2). The number of individuals caught remained relatively stable over the study periods at all grids except Balban which experienced a substantial decline over the 12 months.

Table 4.2.1. The number of trap nights for trapping sessions at the WCRP PCS grids.

Session	Keninup	Balban	Warrup	Boyicup	Winnejup	Karakamia
Jul-06	100	100	100	100	100	
Aug-06	75	100	124	194	196	
Sep-06	148	148	148	196	148	196
Oct-06	120	120	120	120	120	
Nov-06						120
Dec-06	120	120	120	120	120	
Jan-07						120
Feb-07	120	120	120	120	120	
Mar-07						90
Apr-07	120	120	120	120	120	
May-07						120
Jun-07	120	120	120	120	120	
Jul-07						90

Table 4.2.2. Trap effort and number of woylie individuals caught for each trap session at WCRP PCS grids.

Session	Keninup	Balban	Warrup	Boyicup	Winnejup	Karakamia
Jul-06	21	11	14	1	2	-
Aug-06	25	12	14	1	3	-
Sep-06	29	10	15	2	6	47
Oct-06	32	13	27	2	2	-
Nov-06	-	-	-	-	-	45
Dec-06	27	7	14	2	1	-
Jan-07	-	-	-	-	-	40
Feb-07	36	4	18	2	1	-
Mar-07	-	-	-	-	-	42
Apr-07	33	5	18	2	2	-
May-07	-	-	-	-	-	44
Jun-07	25	3	13	0	2	-
Jul-07	-	-	-	-	-	42

4.2.3.3. Population abundance

The total capture rates (all species combined) varied considerably between sites: Keninup (46-91%), Balban (32-75%), Warrup (60-77%), Boyicup (13-52%), Winnejup (4-61%), Karakamia (85-93%).

Woylie capture rates were substantially greater than koomal and other species at Keninup (Figure 4.2.1). The capture rates of woylies and koomal were initially similar at Balban but koomal increased substantially in the same period that woylies declined (Figure 4.2.2). The capture rates of woylies and koomal remained similar and relatively stable at Warrup throughout the study period (Figure 4.2.3). Koomal capture rates increased substantially over time while woylie and other captures remained very low at both Boyicup (Figure 4.2.4) and Winnejup (Figure 4.2.5). Woylie capture rates at Karakamia remained at extremely high levels (73-80%) throughout the study while koomal and other species were consistently seldom captured (Figure 4.2.6).

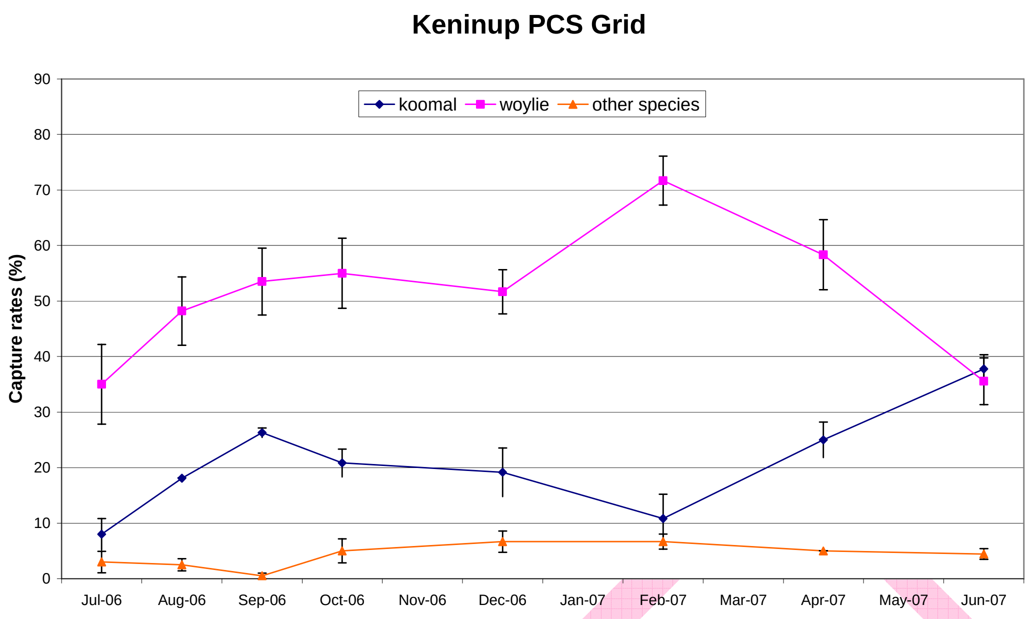

Figure 4.2.1. Capture rate for woylies, koomal and other species at the Keninup Population Comparison Study grid in the Upper Warren region.

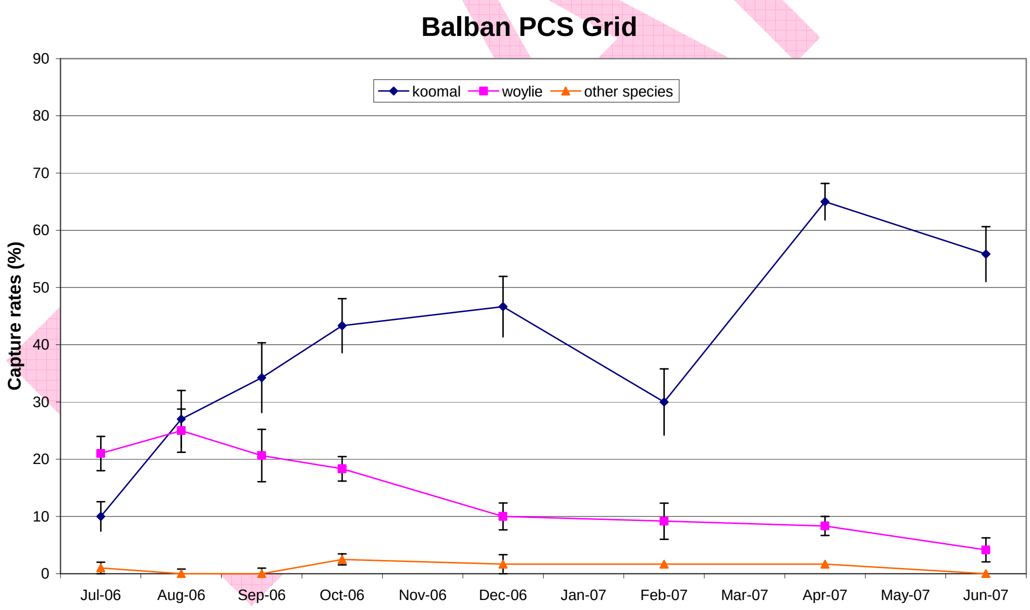

Figure 4.2.2. Capture rate for woylies, koomal and other species at the Balban PCS grid in the Upper Warren region.

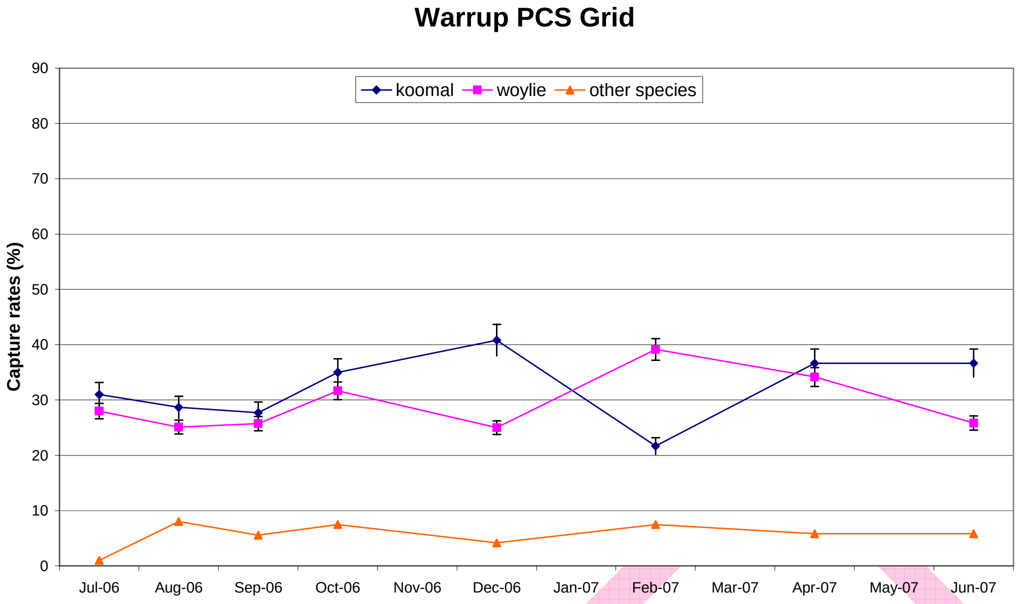

Figure 4.2.3. Capture rate for woylies, koomal and other species at the Warrup PCS grid in the Upper Warren region.

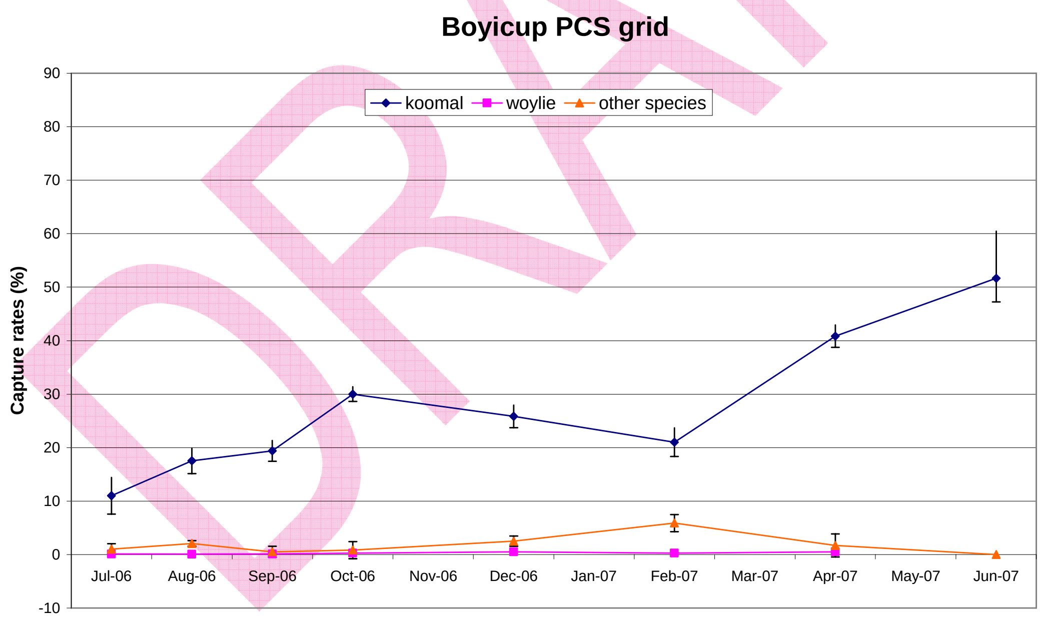

Figure 4.2.4. Capture rate for woylies, koomal and other species at the Boyicup PCS grid in the Upper Warren region.

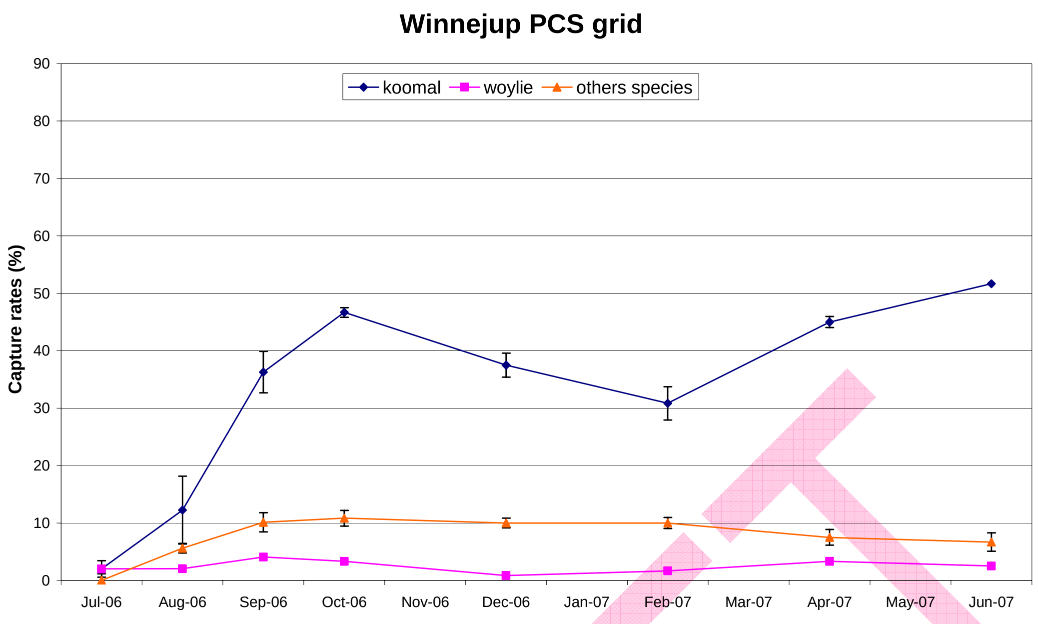

Figure 4.2.5. Capture rate for woylies, koomal and other species at the Winnejup PCS grid in the Upper Warren region.

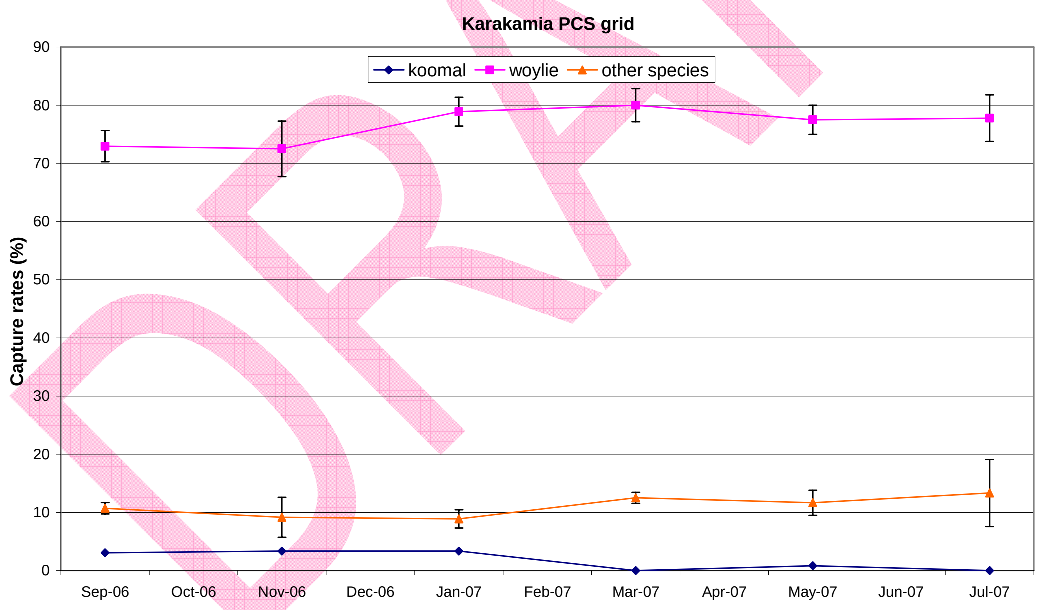

Figure 4.2.6. Capture rate for woylies, koomal and other species at the Karakamia PCS grid in the Upper Warren region.

4.2.3.4. Animal weight

Adult woylies at Karakamia were significantly smaller than Upper Warren sites (means of 1070 g and 1368 g, respectively). Other biometric attributes remain to be examined and compared between PCS sites, including size and animal condition (i.e. a function of size and mass).

The temporal variation in mean weights of woylies for the Karakamia reference grid (Figure 4.2.7) varied slightly, with May 2007 showing a small reduction in the mean weights. There were no significant gender differences in adult weights (female adults mean = 1070 g, range = 1003 g - 1113 g; and male adults mean = 1071 g, range = 1005 g -1112 g).

The mean weights of adult woylies for the Upper Warren PCS grids remained relatively stable over time (Figure 4.2.8). Adult females tended to be heavier, although this was not substantial or consistent (female adults mean = 1385 g, range = 1350 g -1437 g; and male adults mean = 1360, range = 1320 g -1395 g).

The mean weights of adult woylies varied between Upper Warren PCS sites (Figure 4.2.9), however caution is required given that repeat measurements of the same individuals have had a disproportionate influence in deriving the mean values for sites with low capture rates (i.e. Boyicup and Winnejup) – this will be addressed in subsequent analyses in which average weights per individual will be used in deriving the mean (i.e. a mean derived from independent measures).

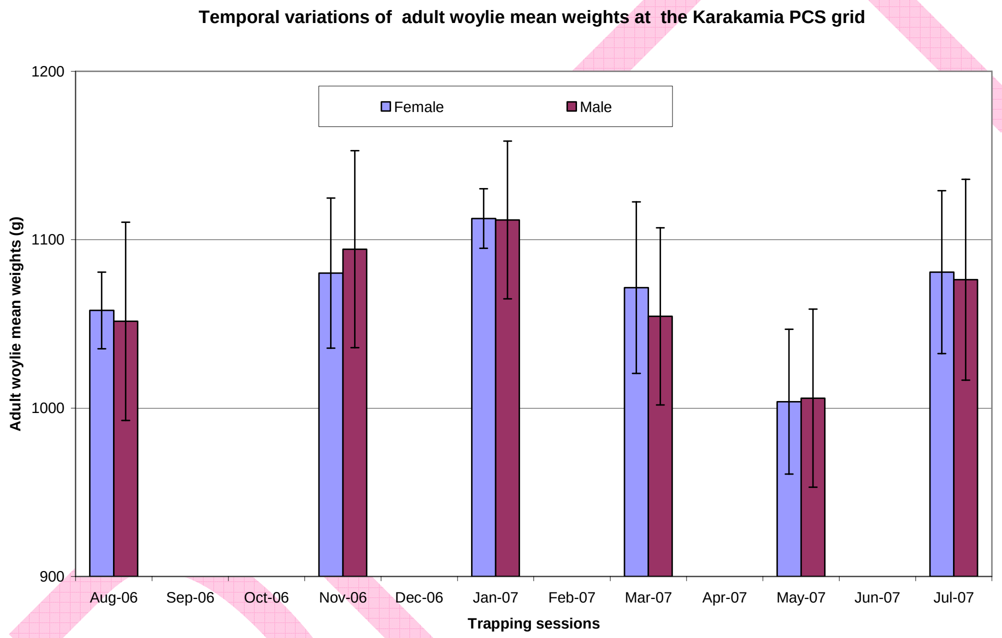

Figure 4.2.7. Temporal variations of adult woylie mean weights at the Karakamia PCS grid

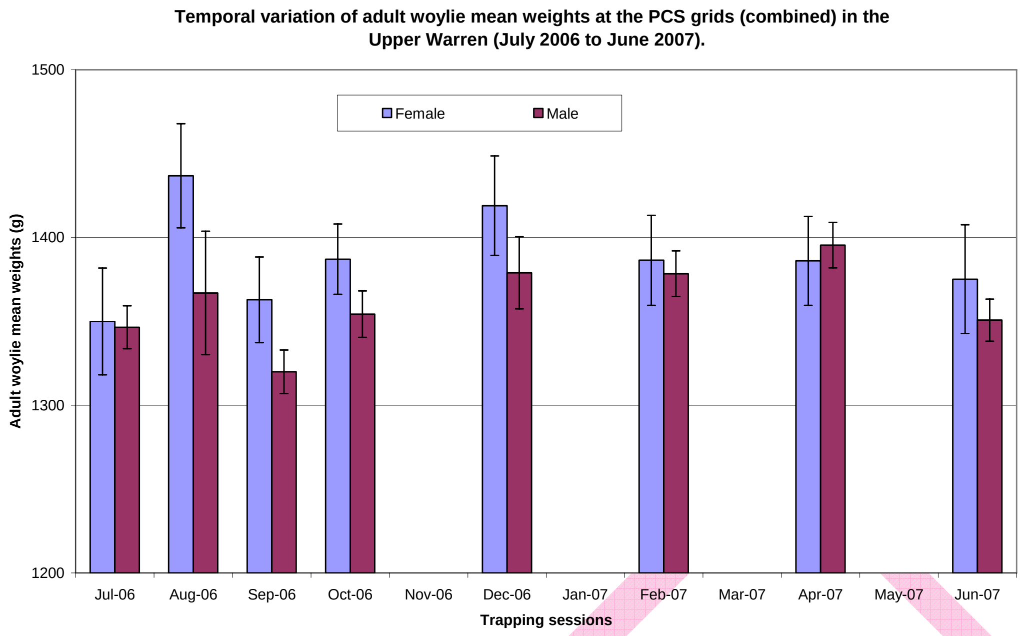

Figure 4.2.8. Temporal variation of adult woylie mean weights at the PCS grids (combined) in the Upper Warren (July 2006 to June 2007).

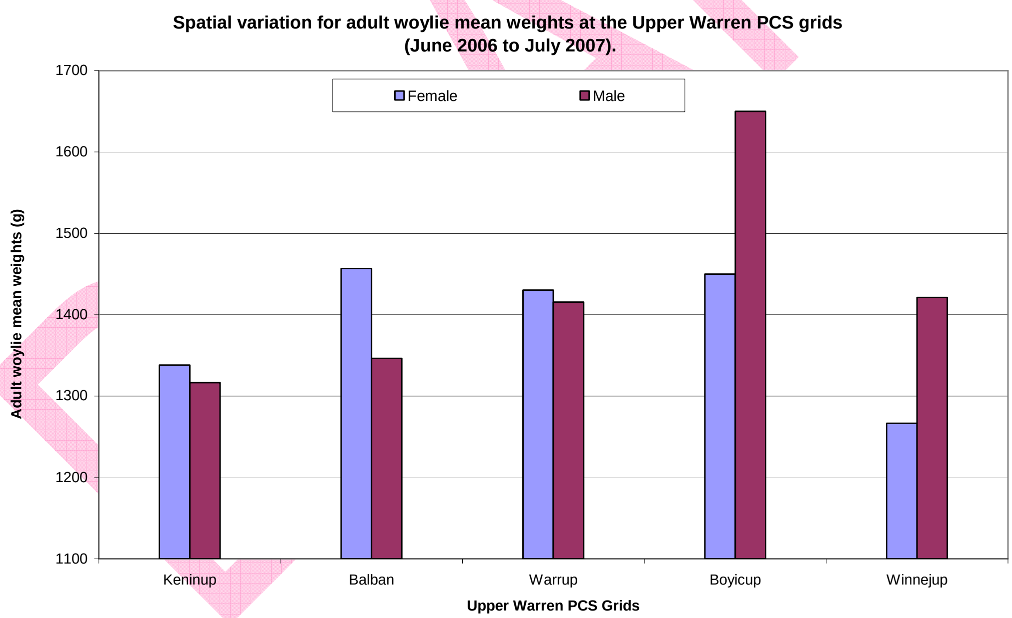

Figure 4.2.9. Spatial variation for adult woylie mean weights at the Upper Warren PCS grids (June 2006 to July 2007).

4.2.3.5. Sex ratio

The ratio of captured adult males to adult females were on average 2.0, 2.7 and 1.6 for Keninup, Balban, Warrup, respectively (Table 4.2.3). This was substantially different from the female bias in Karakamia captures (male/female ratio=0.7; Table 4.2.4). The samples sizes at Boyicup and Winnejup were too small to reasonably assess sex ratios and to compare whether the sex ratios at the 'woylie declined' sites were any different to the more abundant sites where declines had not yet occurred.

Sex ratios generally remained relatively stable over time except where sample sizes were particularly small (Table 4.2.3). The reduction of sample size at Balban over time makes it particularly difficult to confidently determine whether the sex ratio changed in association with woylie declines at this site.

Table 4.2.3. The sex ratio of captured woylies (male/female) at the PCS grids in the Upper Warren.

Grid		Jul-06	Aug-06	Sep-06	Oct-06	Dec-06	Feb-07	Apr-07	Jun-07
Keninup	**Ratio M/F**	2.5	2.6	2.2	1.7	1.5	1.8	1.4	2.1
	n	*21*	*25*	*29*	*32*	*27*	*36*	*33*	*25*
Balban	**Ratio M/F**	2.7	2.0	2.3	1.6	2.5	-	4.0	2.0
	n	*11*	*12*	*10*	*13*	*7*	*4*	*5*	*3*
Warrup	**Ratio M/F**	1.8	1.8	1.5	1.7	1.8	1.6	1.6	0.9
	n	*14*	*14*	*15*	*27*	*14*	*18*	*18*	*13*
Boyicup	**Ratio M/F**	-	-	1.0	1.0	1.0	1.0	1.0	-
	n	*1*	*1*	*2*	*2*	*2*	*2*	*2*	*0*
Winnejup	**Ratio M/F**	1.0	2.0	5.0	-	-	-	-	-
	n	*2*	*3*	*6*	*2*	*1*	*1*	*2*	*2*

Table 4.2.4 The sex ratio of woylies (male/female) for the Karakamia PCS grid.

Grid		Sep-06	Nov-06	Jan-07	Mar-07	May-07	Jul-07
Karakamia	**Ratio M/F**	0.57	0.61	0.74	0.83	0.69	0.62
	n	*47*	*45*	*40*	*42*	*44*	*42*

4.2.3.6. Age demographics

Subadults comprised an average 4.2% (subadult:adult ratio = 0.06) of the independent woylie individuals trapped in any one session at Keninup (Table 4.2.5). Warrup trapped only 2 subadults (February 2007). Balban, Boyicup and Winnejup recorded no subadult captures (Table 4.2.5). Karakamia had an average of 4.6% subadults within the independent woylie population (Table 4.2.6), however, no subadults were captured in January, March or May 2007.

Given the very low incidence rates of captured subadults and the limited sample sizes it is not possible to determine whether there is a statistically significant difference in the age demographics of these populations, in particular whether the absence of subadults at Balban is significantly different from populations that have not undergone decline.

Table 4.2.5. The prevalence of adults, subadults, and juvenile/infants combined, for the PCS grids in the Upper Warren region.

Grid		Jul-06	Aug-06	Sep-06	Oct-06	Dec-06	Feb-07	Apr-07	Jun-07	Ave	SE
Keninup	**Adult**	*21*	*25*	*29*	*32*	*27*	*36*	*33*	*25*	-	-
	Subadult	*1*	*1*	*2*	*2*	*2*	-	*2*	-	-	-
	Juv./Infant	*8*	*8*	*8*	*12*	*13*	*11*	*14*	*8*	-	-
	S:A %	4.5	3.8	6.5	5.9	6.9	0.0	5.7	0.0	**4.17**	**0.01**
Balban	**Adult**	*11*	*12*	*10*	*13*	*7*	*4*	*5*	*3*	-	-
	Subadult	-	-	-	-	-	-	-	-	-	-
	Juv./Infant	*4*	*1*		*5*		*1*	*1*	*1*		
	S:A %	-	-	-	-	-	-	-	-	-	-
Warrup	**Adult**	*14*	*14*	*15*	*27*	*14*	*18*	*18*	*13*		
	Subadult	-	-	-	-	-	*2*		-	-	-
	Juv./Infant	*6*	*2*	*7*	*8*	*1*	*1*	*6*	*5*	-	-
	S:A %	0	0	0	0	0	0.10	0	0	**0.01**	**-**
Boyicup	**Adult**	*1*	*2*	*2*	*2*	*2*	*2*	*2*	-	-	-
	Subadult	-	-	-	-	-	-	-	-	-	-
	Juv./Infant	*0*			*2*	*1*	*1*	*1*	-	-	-
	S:A %	-	-	-	-	-	-	-	-	-	-
Winnejup	**Adult**	*2*	*3*	*6*	*2*	*1*	*1*	*2*	*2*	2.38	-
	Subadult	-	-	-	-	-	-	-	-	-	-
	Juv./Infant	*1*	*1*	*1*	-	-	-	-	-	-	-
	S:A %	-	-	-	-	-	-	-	-	-	-

Table 4.2.6. The prevalence of adults, subadults, and juvenile/infants combined, for the PCS grid at Karakamia

		Sep-06	Nov-06	Jan-07	Mar-07	May-07	Jul-07	Ave	SE
Karakamia	**Adult**	*47*	*45*	*40*	*42*	*44*	*42*		
	Subadult	*6*	*6*				*2*		
	Juv./Infant	*23*	*9*	*1*	*12*	*19*	*23*		
	S:A %	11.3	11.8	0.0	0.0	0.0	4.5	**4.61**	**4.77**

4.2.3.7. Reproduction

The proportion of adult females with pouch-young remained high at all Upper Warren sites (Table 4.2.7). The pouch-young records were absent in some of the Warrup data in particular. The proportion of adult females with pouch-young throughout the study ranged from 76% to 100% (average 89%).

At Karakamia the proportion of adult females with pouch-young varied over time. The proportion was initially high in Spring 2006, and was at its lowest in January 2007 (4%) before increasing through autumn and winter 2007 (Figure 4.2.10).

Table 4.2.7. The presence/absence of pouch-young (PY) with adult female woylies captured at the PCS grids in the Upper Warren region.

		Jul-06	Aug-06	Sep-06	Oct-06	Dec-06	Feb-07	Apr-07	Jun-07
Keninup	**F with PY**	6	6	9	12	11	12	14	8
	F no PY	-	1	-	-	-	1	-	-
	F no record	-	-	-	-	-	-	-	-
Balban	**F with PY**	3	3	2	5	2	2	-	1
	F no PY	-	-	-	-	-	-	-	-
	F no record	-	1	1	-	-	-	-	-
Warrup	**F with PY**	5	3	4	6	2	7	6	5
	F no PY	-	1	-	1	1	-	1	-
	F no record	-	1	1	3	2	-	-	2
Boyicup	**F with PY**	-	-	1	1	1	1	1	-
	F no PY	-	-	-	-	-	-	-	-
	F no record	-	-	-	-	-	-	-	-
Winnejup	**F with PY**	1	1	1	-	-	-	-	-
	F no PY	-	-	-	-	-	-	-	-
	F no record	-	-	-	-	-	-	-	-
Total n	**Females**	**15**	**17**	**19**	**28**	**19**	**23**	**22**	**16**
Total %	**F with PY**	**100%**	**76%**	**89%**	**86%**	**84%**	**96%**	**95%**	**88%**

Table 4.2.8. The presence/absence of pouch-young (PY) with adult female woylies captured at the Karakamia PCS grid

		Sep-06	Nov-06	Jan-07	Mar-07	May-07	Jul-07
Karakamia	**F+ PY**	25	14	1	13	18	23
	F - PY	6	12	22	10	9	6
	F No rec						
Total n	**Females**	**31**	**26**	**23**	**23**	**27**	**29**
Total %	**F with PY**	**81%**	**54%**	**4%**	**57%**	**67%**	**79%**

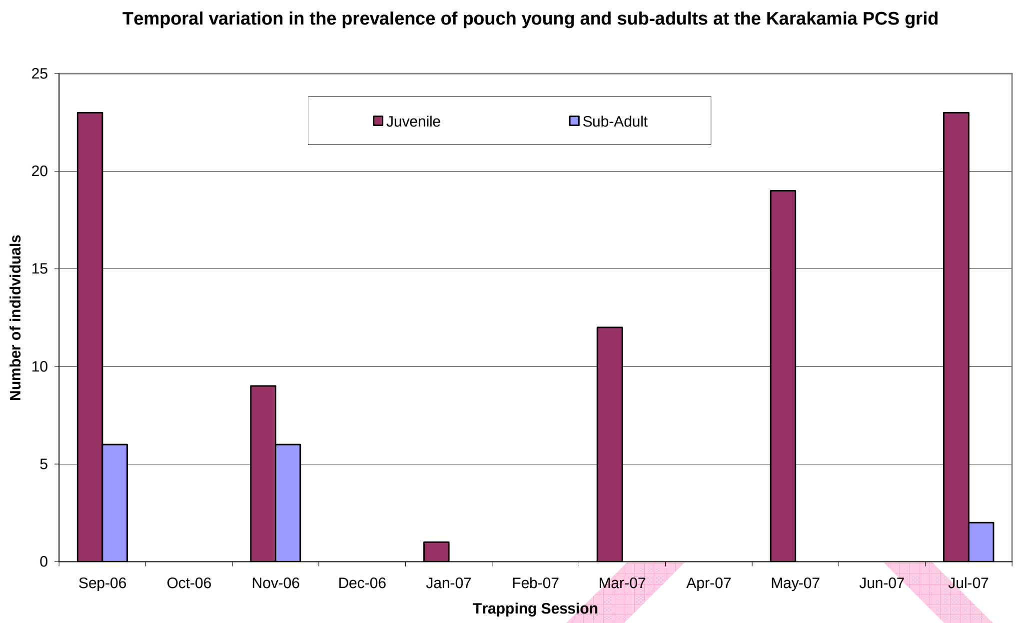

Figure 4.2.10. Temporal variation in the prevalence of pouch-young and subadults at the Karakamia PCS grid.

Pouch-young survival

Juvenile woylies were implanted with Trovan microchips (PIT tags) where possible and appropriate. Only fully furred juveniles (i.e. still dependent on their mother) were implanted for welfare considerations and implanting was only conducted by individuals experienced and trained to do so (i.e. AW, JW, MM and CW). As a result, only three juveniles were implanted and successfully released with their mother at Keninup, two at Balban and one at Warrup. One of these juveniles was subsequently recaptured as an adult at Keninup (not on the grid where originally located but on the transect nearby). An additional two juveniles that were implanted at Keninup and one from Balban were not successfully released at or shortly after implanting and were taken into care.

4.2.4. Discussion

4.2.4.1. Relative woylie abundances

The spatial and temporal patterns in the relative abundances of the woylie derived from the Population Comparison Study (PCS) grids (i.e. site level) were entirely consistent with the trapping data derived from the Upper Warren Fauna Monitoring transects (Chapter 2) and the woylie activity indices using sandpads (Section 4.4 Predators) that were both operating at larger landscape scales (i.e. forest blocks) within the same areas. Of particular note is the 81% decline in the capture rate of woylies at Balban over the 12-month study period. This was in contrast to the relatively stable temporal trends observed at the last remaining moderate density populations in the Upper Warren (Keninup and Warrup), the previously declined and now low density sites of Boyicup and Winnejup and the persistently high density population at Karakamia.

An associative trend between woylies and koomal was also apparent among the PCS sites. Where moderate densities of woylies persisted (i.e. Karakamia, Keninup and Warrup), koomal capture rates were also stable. Where woylies were either declining (Balban) or had previously declined and remained at low densities (Boyicup and Winnejup), koomal capture rates substantially increased between July 2006 and June 2007. This is consistent with observations at Dryandra (Orell, 2004). At the Upper Warren regional scale, the meta-analysis found no significant association between koomal and woylie population changes over time (Chapter 3 Meta-analysis). A definitive verification of whether there exists any association between these species and the nature of the relationship (i.e. cause or effect), would be useful evidence that would assist the diagnosis of woylie declines.

4.2.4.2. Sex ratio

The substantial differences in the sex ratios observed in the moderate density populations in the Upper Warren (male biased) compared with the high density population at Karakamia (female biased) is particularly striking. It is not possible to reasonably infer directly from trap capture data what the *actual* sex ratio within a population may be because of the gender biases in trappability and the influences that trapping methodology has on these biases (Wayne *et al.,* in press). The identical trapping methodology between the PCS grids eliminates any influence that this may have on the sex ratio of captured individuals, however, the extent to which differences in animal density may confound direct comparability between sites is unknown. Despite these constraints, it is remains likely that real and substantial differences exist between Karakamia and the Upper Warren moderate density populations.

No temporal changes in the sex ratio were detected as the Balban population declined. However, the small sample sizes fundamentally limited the ability to confidently detect anything but the most dramatic of changes (i.e. poor statistical power *e.g.* Taylor and Gerrodette, 1999). Similarly the same inherent problem arises when attempting to compare the 'declined', low density populations with the moderate density sites. These limitations are common to all of the demographic attributes examined here, and are symptomatic of the challenges to generate sufficient data for statistical inference from animals that may be rarely encountered and/or become increasingly so over time (e.g. Caughley, 1994; Caughley and Gunn, 1996).

4.2.4.3. Recruitment potential – prevalence of pouch-young and subadults

The high proportion of adult females encountered with pouch-young (i.e. average of 89% in the Upper Warren and 57% in Karakamia) was consistent with earlier findings (89% in Perup; Christensen, 1980). Not withstanding the interpretive constraints of small sample sizes (already discussed) it is likely that reproductive rates remained consistently high among adult females at the Upper Warren PCS grids (i.e. including declining and declined populations). This was different to Karakamia, which varied substantially over time. The temporal variation in the prevalence of subadults in the Karakamia woylie captures is consistent with a temporal lag in the presence of pouch-young. Data from multiple years would be required to verify that this temporal variation is a likely seasonal breeding pattern (i.e. reduced or no breeding in the hotter/drier months). This contrasts the apparent aseasonal breeding within the Upper Warren region, which is consistent with previous research in the area (Christensen, 1980; Wayne unpublished data).

The three principle factors that may account for the low prevalence of subadults in a captured population include;

- The relatively short time that independent individuals remain sexually immature (i.e. subadult woylie for 30-60 days) compared with the time spent in the pouch (100-110 days) and normal life expectancy (5-6 years) (Christensen, 1980; Seebeck and Rose, 1989) (i.e. relates to probability of capture within a short time period)
- differential mortality rates of subadults, and
- possible trappability differences

High mortality rates of woylies around the age of independence is a likely significant contributor to the low prevalence of subadults, however, more sophisticated analyses and population modeling are required to quantify the relative mortality rates of woylie life stages. Nonetheless, the observations in this study are at least broadly consistent with previous research of woylies in the Upper Warren which found that pouch-young survival was relatively high (82-91%) but low after pouch emergence with only 11-15% of young surviving through to adulthood (Christensen, 1980).

No subadults were detected within the declining woylie population at Balban (despite moderate prevalences of infants and juveniles), or at the two declined populations at Boyicup and Winnejup. Similarly, Warrup recorded only two subadults. Determining whether there has been a reduction in the prevalence of subadults associated with the woylie declines is not possible using the PCS dataset alone, given the limitations of the data (small number of animals captured and low prevalence rates of subadults). Pooling of the PCS data with compatible monitoring data may however, provide enough data to examine whether there are any differences in the prevalence and potential recruitment rate of immature animals associated with the declines.

Challenges associated with measuring juvenile recruitment and survival has limited that ability to look at this potentially important component of demographics in more detail. The sample sizes obtained in this study of fully-furred juveniles suitable for PIT implants were insufficient for statistical inference. Substantially greater trapping effort (frequency and extent) to increase sample sizes would be required to overcome this at the moderate-density PCS sites, even more so for the low-density sites. Subsequent surveys would need to be sufficiently extensive to account to some extent for the dispersal of subadults away from their natal range. The use of radio-telemetry (usually via neck collars or tail fixtures) is also limited by the capacity to accommodate rapid growth of juveniles and subadults, but does more readily overcome issues associated with tracking the fate of dispersing animals. A third complementary or alternative approach may indirectly infer the survival or recruitment rate by comparing the relative prevalence of pouch-young, juveniles, subadults and new adults. Having considered the necessary assumptions (e.g. differences in capture probabilities), the differences in the prevalence rates can be used to derive an estimate of survival. This remains to be fully explored with the data currently available.

4.2.5. Future work

The main priority for the PCS investigation in woylie demographics is to develop and complete the data analysis in preparation for publication in a peer-reviewed paper. This will include;

- Complete more rigorous analyses of the demographic attributes initially explored and discussed in this report
- Analyse the biometric data to investigate demographic differences in animal size, weight, condition, etc over space and time, and in relation to woylie declines
- Analyse the trap capture data to investigate population differences in population turnover and recruitment (i.e. incidence of new animals over space and time)

The field work associated with the PCS of woylie demographics ceased (indefinitely) in June 2007. The continuation or development of further demographic research will be reviewed as part of the greater review of the Woylie Conservation Research Project.

- The vegetation assessment of PCS trapping sites for the purposes of density and demographic comparisons and relatedness of data is still to be completed.

4.2.6. Conclusion

Some of the key preliminary results from an initial exploration of the demographic data include;

- Balban underwent an 81% decline in woylie capture rates in the 12 months from June 2006. Capture rates at all of the PCS grids were consistent with other woylie data sets.
- Koomal capture rates increased at grids where woylies declined (Balban) or had previously declined (Boyicup and Winnejup) and remained relatively stable at the stable moderate-high woylie density grids (Karakamia, Keninup, Warrup).
- Small sample sizes from low density and declining populations inherently limits the power of statistical inference to determine if there are significant differences in the demographic attributes between woylie populations at various stages of decline. This emphasizes the importance of having sufficient resources available to support the increased effort required to collect sufficient data for analysis.
- There is no evidence that there are substantial changes in reproduction associated with Upper Warren woylie declines (inferred from prevalence of pouch-young).
- It remains uncertain whether the survival of offspring through to independence (subadult) and sexual maturity (adult) differs in association with woylie declines.
- The Karakamia woylie population in recent times is remarkably different on a number of accounts including, especially high woylie densities, female bias in sex ratio of captured individuals, likely seasonal breeding, and substantially smaller mean weights of adult males and females.
- More formal and thorough analyses are required before evidence from the demographics of woylies can be used to confidently investigate for clues as to the mechanics and possible causes of decline.

4.2.7. References

Caughley, G. 1994. Directions in Conservation Biology. Journal of Animal Ecology **63**:215-244.

Caughley, G. C., and A. Gunn 1996. Conservation Biology in Theory and Practice. Blackwell Science, Cambridge.

Christensen, P.E.S. 1980. The biology of *Bettongia penicillata* Gray, 1837, and *Macropus eugenii* Desmarest, 1817, in relation to fire, Forests Department of Western Australia, Bulletin **81.**

Orell, P. 2004. Fauna monitoring and staff training: Western Shield review - February 2003. Conservation Science Western Australia **5**:51-95.

Seebeck, J., and R. W. Rose. 1989. Potoroidae. Pages 716-739 in D. W. Walton, and B. J. Richardson, editors. Fauna of Australia. Mammalia. Australian Government Publishing Service, Canberra.

Taylor, B. L., and T. Gerrodette. 1993. The Uses of Statistical Power in Conservation Biology - the Vaquita and Northern Spotted Owl. Conservation Biology **7**:489-500.

Wayne, A. F. 2006. Interim assessment of the evidence for a recent decline in woylie abundance in south-western Australia. Pages 1-28. CALM, Perth, Western Australia.

Wayne, A. F., J. Rooney, K. D. Morris, and B. Johnson. in press. Improved bait and trapping techniques for chuditch (*Dasyurus geoffroii*): overcoming reduced trap availability due to increased densities of other native fauna. Conservation Science.

4.2.8. Acknowledgements

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4.3. Survival and mortality

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Abstract

A woylie 'search and rescue' exercise (June 2006) aimed to recover sick and dead woylies that might provide evidence for the recent declines. A total of 15 searches within four forest blocks in the Upper Warren (two supporting moderate woylie densities and two where woylies recently declined) involved 66 DEC personnel and volunteers and resulted in the recovery of 17 woylie bodies (all in advanced stages of decomposition) and no sick woylies, among other finds.

The survival and mortality of 58 radio-collared woylies were examined at the Upper Warren population comparison study sites, July 2006 – June 2007. Monitoring of the mortality-sensitive radio-collars was generally conducted every weekday. Radio-collars were removed from 16 individuals (two were later recollared) during the study and collars fell off another three animals (one of which was subsequently recollared). A total of 21 mortality events were observed. Forensic evidence collection included the body site, animal remains (in the field and lab), necropsy (when possible), DNA, and forensic odontology.

This study demonstrates that the woylie declines result in part, at least, from mortality and not loss through emigration. Survival was substantially reduced while the declines were occurring at Balban relative to other sites not presently declining or having previously declined. Predation/scavenging is proximately associated with 20 of the 21 mortalities observed. Preliminary evidence indicates that cats were primarily associated with 13 woylie mortalities, four with raptor and three with fox. Chuditch were implicated at nine carcasses (DNA and odontology) but were generally considered secondary scavengers based on the field evidence. Based on the available evidence it is hypothesized that the ultimate factor(s) related to woylie mortalities (and population declines) is not predator related, rather predators are exploiting a prey made more vulnerable by other factor(s).

4.3.1. Introduction

Population decline may result from reduced recruitment (i.e. reproduction and/or immigration) and/or increased loss (i.e. mortality and/or emigration) of individuals from the population. The early results from the situation assessment to determine if woylies had declined in the Upper Warren (Wayne, 2006; Wayne *et al.,* 2006) demonstrated that the woylie declines were rapid (25-90% per annum). These rates of decline were greater than what might be possible if recruitment failure was complete (i.e. assuming woylies lived for 5-6 years the rate of decline due to complete reproductive recruitment failure could be no greater than 16-20% per annum). It was therefore clear that the woylie declines resulted, in part at least, from an *increased loss* of individuals from the population. As a result of this important finding, this study focused on the potential factors associated with the loss of woylie individuals from the Upper Warren Population Comparison Study (PCS) sites. A good understanding of these factors was considered critically important to providing clues, if not direct evidence, of the principle cause(s) behind the woylie declines.

The principal objectives of this study were to determine if there were differences between woylie populations at difference stages of decline with respect to i) survival, and ii) mortality.

4.3.2. Methods

4.3.2.1. Woylie 'search and rescue' area searches

A woylie 'search and rescue' exercise on 17 June 2006 aimed to recover sick, moribund and dead woylies that might provide evidence for the recent declines. Two forest blocks were selected that had most recently undergone substantial declines in woylie numbers (Chariup and Yendicup).

Another two forest blocks were surveyed that still supported moderate densities of woylies (Balban and Keninup). Area searches were conducted using similar methods to those used by the SES and Police to search for lost people, bodies and/or evidence. SES volunteers provided support in the execution of these searching methods. A total of 66 DEC personnel and volunteers, including year 12 students from Bunbury Cathedral Grammar School were organised into five search teams. Each team conducted three searches, one each at Chariup and Yendicup, and another at either Balban or Keninup. Each search was conducted for around 60 minutes. A wildlife veterinarian team from Perth Zoo was on site to manage any sick or moribund woylies that were recovered.

4.3.2.2. Radio-collared cohorts

Cohorts of radio-collared individuals were used as the principle means of investigating the survival and mortality of woylies. Beginning in July 2006, cohorts within each of the five Upper Warren PCS woylie populations were collared with two-stage mortality-sensitive radio-transmitters (Biotrack, U.K.). Using the trapping activities on the grids associated with the woylie demographics study (Section 4.2 Demographics), adult individuals were selected for collaring with the objective of getting at least six males and six females at each site. Only individuals from these grids were selected for radio-collaring to maximise the likelihood of repeat captures for close examination and because of the substantial logistic benefits of having the study subjects in close proximity to each other. Young subadult animals were not radio-collared because of equipment limitations and welfare considerations of having fixed-diameter collars on growing animals.

At the time of radio-collaring, individuals were given full clinical health checks, and extensive sampling for disease and dietary analyses (blood, scats, ectoparasites, and ear tissue for DNA) to develop as complete a health profile as possible. Subsequent recaptures of these individuals during the PCS demographics study and Upper Warren Fauna Monitoring (Chapter 2) involved routine detailed health checks and the non-invasive sampling of faeces and ectoparasites for disease screening. Repeat sampling of blood was predominantly limited to approximately 6 months (re-collaring and cohort supplementation) and 12 months (collar removal) after first collaring, and individuals opportunistically captured along the Upper Warren Fauna Monitoring transects (October-November 2006 and March-April 2007).

The monitoring of the radio-transmitters commenced immediately after the first animals were collared. The objective was to monitor all animals every weekday over a 12-month period. When this was logistically not possible due to poor weather conditions or resource limitations, every effort was made to ensure no more than two days elapsed between monitoring events. The approximate location of the animals were recorded, particularly those instances when individuals were not located on the PCS demographics study grids. The monitoring of the radio-transmitters was preferentially conducted by fixed-wing aircraft principally because of the efficiency and economic advantages. Monitoring from the ground was done when it was possible to be associated with other WCRP field work (e.g. trapping and predator sandpad monitoring). No fine-scale animal movement or habitat use data were collected from radio-collared woylies given the resource limitations and priorities.

All mortality events were intensively investigated to collect as much forensic evidence as possible. This included evidence from the site where the body and/or radio-collar were recovered, animal remains (examined in the field and laboratory), necropsy (when possible, by the Duty Pathologist at Murdoch University), forensic DNA (Dr Oliver Berry, University of Western Australia), and forensic odontology (Denice Higgins, University of Adelaide). A more complete account of the methodology associated with this study is provided in the WCRP Operations Handbook (Volume 3)

4.3.3. Results

4.3.3.1. Woylie 'search and rescue' area searches

The search effort from each of five teams (total of 66 people), each completing three one-hour exercises resulted in 9061 people-minutes of searching (Table 4.3.1). The finds included 17 woylie remains, 13 woylie nests and five live woylies (Table 4.3.2). Remains located during the searches were generally in advanced stages of decomposition. No sick or moribund animals or fresh carcasses were located.

Table 4.3.1. The search effort for the woylie 'search and rescue' in the Upper Warren, 17 June 2006.

	Chariup	Yendicup	Balban	Keninup
Woylie capture rate (%; Apr06)	6	8	36	51
% Woylie extant (Apr06)	10	11	54	-
Number of area searches	5	5	3	2
Total search effort (min)	1980	3758	1815	1508
Minutes per woylie body found	495	1879	605	188
Minutes per live woylies	N/A	N/A	N/A	302
Minutes per woylie nests	1980	3758	908	168

* search time (person-minutes) per unit find

Woylie capture rates and % extant sourced from Upper Warren Fauna Monitoring data (April 2006)

Table 4.3.2. Summary of the finds from the woylie 'search and rescue' exercise in the Upper Warren region, 17 June 2006.

Woylie body remains	17
Woylie nests	13
Woylies alive	5
Koomal body remains	3
Chuditch body remains	1
Macropod body remains	8
Bobtail body remains	1
Raptor sign	1 (pellet)
Predator Scats	6
Other animal material	14

* Macropods includes, tammar, western brush wallaby and western grey kangaroo

4.3.3.2. Radio-collared cohorts

Radio-collars were fitted to 58 woylies. The number and gender of woylies collared was limited by the availability of suitable individuals caught at each of the five Upper Warren PCS sites except Keninup (Table 4.3.3). All radio-collared woylies displayed territorial behaviour, each consistently remaining within the same area throughout the period in which they were monitored. There was no evidence of emigration or relocation by any of the radio-collared adult woylies.

Mortalities for the period July 2006 to June 2007 totalled 21 (Table 4.3.3). Six deaths occurred at Keninup, Balban had eight, Warrup had three, Boyicup one and Winnejup three.

Because of skin lesions associated with the radio-collar, 16 were removed to ensure there was no compromise to the welfare of the woylies (or the integrity of the study and its results). Three collars fell off woylies when the epoxy resin joining the brass collar to the radio-transmitter separated.

At completion of the study, 17 collars were recovered from live woylies. One collar and animal were not recovered after the radio-signal was lost at the end of the radio-telemetry monitoring and trapping efforts did not managed to relocate the individual.

Most collars were fitted in July-August 2006. Most mortality events occurred August to November 2006 (Figure 4.3.1). The radio-collared cohorts were then supplemented in December 2006 (12 in Keninup and two in Balban). This included the recollaring of three individuals whose minor neck lesions had completely healed after the earlier removal of their collar. Those collars removed in May and June 2007 were removed at the completion of the project.

Table 4.3.3. Summary details of the radio collared woylies at the PCS grids in the Upper Warren region.

PCS Grid		Collars Fitted	Collars removed during study	Collars removed at end of study	Collars broken	Fate Unknown	Animal Mortality
Keninup	Male	13	4	5	1		3
	Female	14	2	7	1	1	3
	Total	**27**	**6**	**12**	**2**	**1**	**6**
Balban	Male	8	3				5
	Female	4	1				3
	Total	**12**	**4**				**8**
Warrup	Male	6	3		1		2
	Female	5	2	2			1
	Total	**11**	**5**	**2**	**1**		**3**
Boyicup	Male	2	1				1
	Female						
	Total	**2**	**1**				**1**
Winnejup	Male	5		3			2
	Female	1					1
	Total	**6**		**3**			**3**
All Grids	Male	**34**	**11**	**8**	**2**		**13**
	Female	**24**	**5**	**9**	**1**	**1**	**8**
	Total	**58**	**16**	**17**	**3**	**1**	**21**

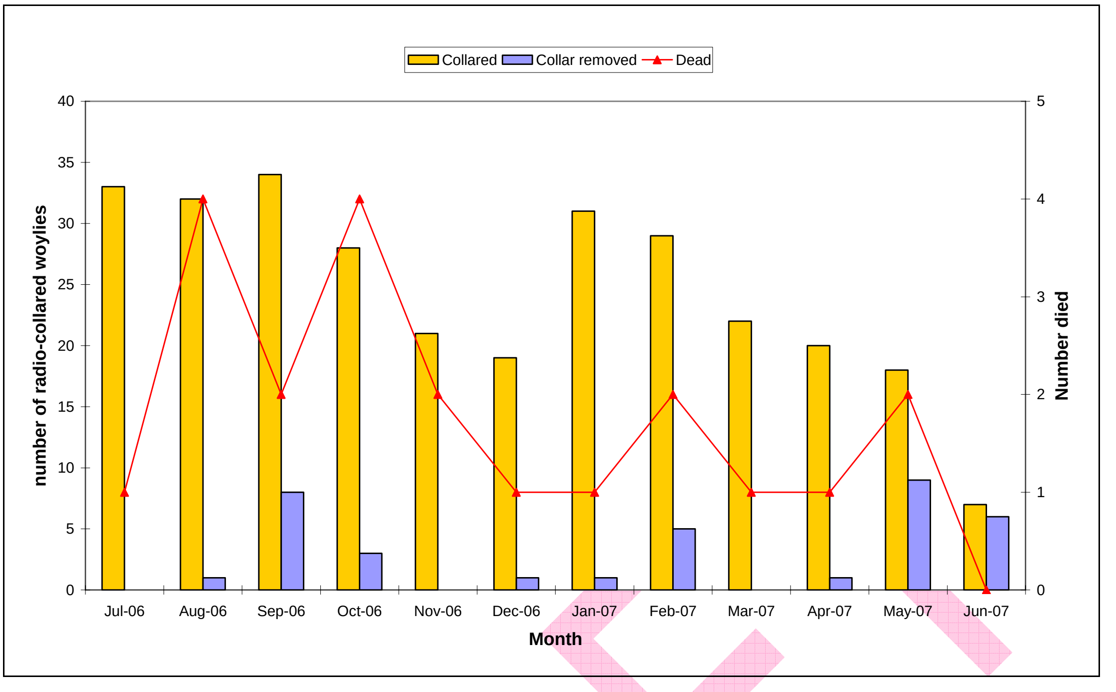
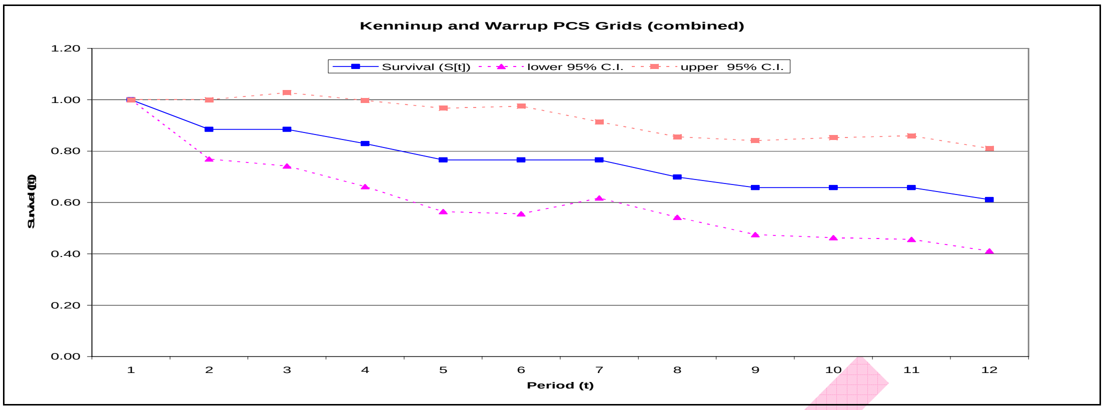


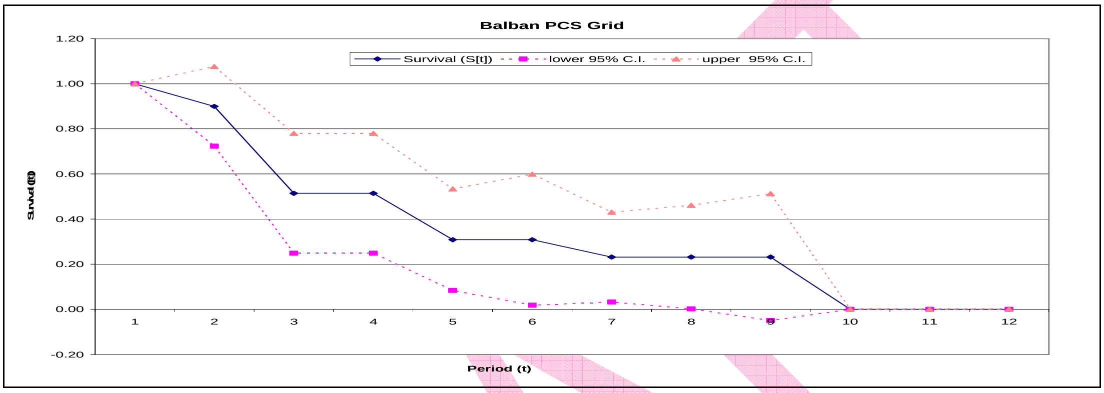
Figure 4.3.1. The fate of radio-collared woylies at the Upper Warren PCS grids (July 2006 to June 2007).

The Kaplan-Meier survival function for the combined sites with relatively stable, moderate densities of woylies (Keninup and Warrup), demonstrated a slower rate of decline compared with Balban (Figures 4.3.2a and 4.3.2b), which was undergoing a significant decline in woylie numbers over the same period (81% decline in capture rates in the same 12 months, see PCS demographics Section 4.2). Woylie survival had declined to zero at Balban by period 10 (30 days per period, '*t*'), compared with 0.66 at the moderate density sites. The mortality rate of the radio-collared cohort at Balban was approximately four times greater than at Keninup (i.e. 0.37% mortality per day versus 0.11% per day respectively). The survival function of the combined sites which declined, now stable, low density woylie populations (Boyicup and Winnejup) had particularly large 95% confidence intervals given the very small cohort sizes (Figure 4.3.2c).

4.3.2a



4.3.2b



4.3.2c

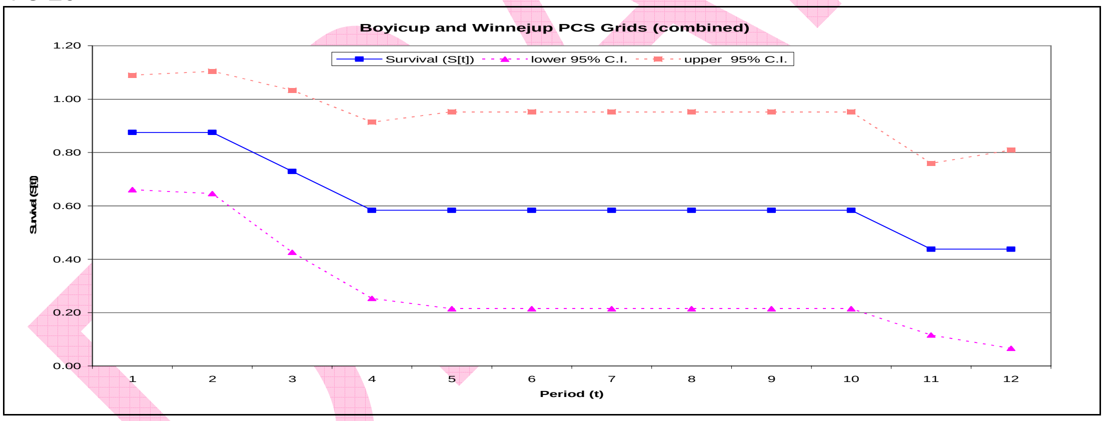


Figure 4.3.2. The Kaplan-Meier survival function for the a) combined moderate-density woylie populations at Keninup and Warrup, b) transitional population at Balban, and c) combined declined, now low-density populations at Boyicup and Winnejup in the Upper Warren region.

Of the 21 mortality events, predation and/or scavenging was evident at all except one body (the inconclusive case from Winnejup; Table 4.3.4). The principle predator or scavenger associated with the mortalities was based on the evidence collected from the location where the body was recovered and the body remains. This evidence was collectively reviewed by most of the authors in a subsequent group workshop. On this basis, the principal predator/scavenger associated with the woylie mortalities was the cat in 13 cases, raptor in four, fox in three and one case remained inconclusive (advanced state of decay and no signs of predation/scavenging). DNA results of samples from mortalities have returned weak amplifications or results and require further work.

DNA and forensics odontology provide opportunities for the collection of other supporting evidence. DNA sampling was conducted on several of the woylies particularly the tooth marks on the radio-collars and bite marks or chewing sites on the woylie bodies. The DNA results are still pending as attempts to improve tests to produce a more conclusive outcome continue. The forensic odontology from the radio-collars in some cases directly supported the primary predator/scavenger but in 10 cases identified a different species. In eight of these cases chuditch were identified by markings on the collar from bodies otherwise thought to have been predated /scavenged by cats. In two cases, field and preliminary DNA evidence identified fox but odontology identified cat marks on the collars.

Two of the radio-collared woylie bodies were sent for necropsy at Murdoch University. Woylie Do 3901/02 was found fresh and almost entirely intact - only the tail was missing with no signs visible of the cause of death. The second necropsy was from a woylie (K 657/658) that died shortly after collaring and before release. There was little opportunity for necropsy on most mortalities because predation/scavenging left very little internal tissues/organs available for examination – most remains consisted of skin, muscle tissue and skeleton only. Occasionally brain material was available for examination.

Necropsy of Woylie DO 3901/02 found moderate autolysis of the spleen, small and large intestines and stomach. Liver, adrenal gland, kidney, lung, heart and brain appeared normal. Multiple puncture wounds were present over the neck and multiple ribs were fractured, consistent with predation.

Woylie K 567/568 pathology reported no distinct cause of death however mild pulmonary oedema and moderate acute subdural / subarachnoid haemorrhage of the brain were present.

Woylie DO 3810/11 was considered a cat predation from physical evidence at the body site. A scat associated with the body was later identified as fox from DNA analysis. This provides an example of the challenges associated between discriminating predator from subsequent scavenging or visits by other animals to the body.

4.3.3.3. Wildlife forensics course and workshop

A 2-day wildlife forensic training course and 1-day applied wildlife forensics workshop was held in October 2006 at the Kensington office of the Department of Environment and Conservation. The course objectives were to improve the understanding and skills of conducting forensic investigations into mortality events and to use this knowledge to develop better forensic investigation protocols. The workshop involved wildlife practitioners and researchers and aimed to compare and share existing methods and experience, and develop where possible, a strategy for collective improvement and learning in wildlife forensic methods. The course was attended by 26 people, principally from the mesopredator research projects, personnel from Species and Communities Branch and Wildlife Officers, and Australian Wildlife Conservancy collaborators.

At the course, evidence collection principles, chain of continuity, documentary procedures and scene evidence photography were presented by Sergeant Mark Reynolds (WA Police Academy). Animal taphonomy, which documents the physical signs and time line of decomposition of a carcass, was outlined by Christopher O'Brien (Centre for Forensic Science, UWA). Forensic entomology presented by Ian Dadour (Centre for Forensic Science, UWA) described the chronological development of fly larvae and beetles to determine time since death. Practical field exercises were also involved.

Table 4.3.4. Predators/scavengers identified from forensic investigations on woylie mortalities.

Site	Date	Woylie ID	Primary Predator / Scavenger	Confidence	Other Evidence: Recent health check	DNA Collar	DNA other	Odontology Collar
Keninup	11/08/2006	K653 K654	Cat	60%		Pending		Chuditch
	19/08/2006	DO3810 DO3811	Cat	50%		Pending	Scat-Fox	Chuditch
	29/08/2006	DO3907 DO3908	Raptor	70%	Fur loss on rump	Pending		Inconclusive
	15/11/2006	DO3901 DO3902	Cat	80%	Skin lesions, heavy lice load	Fox?	No result	Inconclusive
	26/02/2007	DO5011 DO5012	Raptor	75%		Chuditch		Inconclusive
	2/06/2007	DO3781 DO3782	Cat	60%	Heavy tick load, old scar	Pending	Pending	Chuditch
Balban	12/08/2006	DO3921 DO3922	Cat	50%		Pending		Chuditch
	12/09/2006	DO3824 DO3873	Cat	70%		Pending		Chuditch
	29/09/2006	WB2711 WB2710	Cat	70%		Pending		Chuditch
	5/10/2006	WB2749 WB2750	Cat	60%	Heavy lice load	Pending		Cat
	8/11/2006	DO3821 DO3820	Raptor	80%	Heavy lice load	Pending	Body-Fox?	Inconclusive
	4/12/2006	WB2742 DO2743	Cat	60%		Pending		Inconclusive
	30/01/2007	DO7305 DO7306	Cat	60%		Pending	Pending	Cat
	9/04/2007	WB2631 WB2360	Cat	80%		Pending	Pending	Chuditch
Warrup	21/10/2006	DO1869 DO1870	Cat	60%	Fur loss around eyes, old scars	Pending		Chuditch
	26/02/2007	DO3845 DO3846	Fox	90%		Fox?	Pending	
	30/03/2007	DO1842 DO1843	Fox	80%		Fox?	Body-Fox?	Cat
Boyicup	25/10/2006	DO2871 DO2872	Raptor	90%		Pending		Peck mark?
Winnejup	22/07/2006	DO3931 DO3932	Fox	20%		Pending		Inconclusive
	21/10/2006	DO3867 DO3868	Inconclusive	-		Fox?		Inconclusive
	21/05/2007	K276 K277	Cat	60%		Pending		Cat

4.3.4. Discussion

4.3.4.1. Woylie search and rescue

No sick or moribund woylies were detected during the woylie search and rescue (SAR) area searches. The many challenges of detecting unfit woylies include competition with predators (foxes, cats, chuditch, raptors, etc) that are likely to readily and quickly exploit such prey, animal concealment and the significantly lower densities of woylies in areas that had undergone substantial decline. Considering these factors, the use of such a large number of people in the SAR exercise was intended to improve the odds of detecting a sick woylie. Had a sick woylie been found the value to diagnosing the woylie population declines was potentially immeasurable. That no sick or moribund woylies were found does not provide, in itself, any evidence whether or not reduced fitness of woylies is a primary mechanism in the recent and rapid declines because of the associated low probabilities of detection.

The relatively high number of woylie carcasses recovered compared with other abundant mammals (koomal, macropods, etc) is generally consistent with other evidence suggestive of mortality being associated with declines, notwithstanding the potential for differential detection rates of animal remains (e.g. species size and densities, behaviour and ecology). There was no apparent association in the relative detection rates of woylie remains and the woylie population status at the four forest blocks examined, however, a closer examination of the data may be more revealing. Similarly an examination of the forensic evidence associated with the woylie remains may also provide clues related to the cause(s) of the recent declines.

4.3.4.2. Survival and mortality of the radio-collared cohorts

The preliminary results of this study provide direct and compelling evidence that the woylie decline in the Upper Warren is associated with the loss of individuals from the population. Furthermore, this loss has not resulted from adult emigration but through adult mortality. The rate of mortality at Balban, where declines were current, was approximately four times greater than at Keninup, where declines have not yet been apparent.

The rate of mortality was also substantially greater at Balban (approximately three times greater) than at Boyicup/Winnejup (combined) where woylies had recently declined but were at very low and relatively stable densities. The 95% confidence intervals associated with the Kaplan-Meir survival functions indicate that while the mortality rates at Balban are probably significantly greater than at the other sites, there is no significant difference between Keninup and Boyicup/Winnejup – because of the small cohort sizes at the latter sites. This suggests that what may have caused these populations to decline was not still functioning in the same manner after the decline had reduced these populations to less than 5% of their former size. This finding highlights the importance of identifying the agent(s) of decline *while* the declines are occurring, because it seems likely that they may not still be operating and/or as evident after the declines are largely complete. It might also imply that there may be some potential for these populations to recover, if other factors do not maintain the populations at low densities.

While mortality has been directly associated with concurrent woylie declines, this does not indicate in any way whether or not reduction in recruitment is also involved. Further statistical analysis is required to more rigorously determine the survival and mortality differences between these radio-collared cohorts.

4.3.4.3. Factors associated with woylie mortalities

Based on a review of all of the preliminary forensics evidence available to date feral cats appear to be a major predator/scavenger of woylies, particularly in northern Perup at Balban (where declines were current) and Keninup. It is more likely that the cats are predators of woylies, given that it is unusual for cats to scavenge (e.g. Molsher *et al.,* 1999). Cats are also known to be capable of predating similarly-sized prey (e.g. boodie, ngwayir, dalgyte, quenda, chuditch, mala, etc) (e.g. Christensen and Burrows, 1994; Gibson *et al.,* 1994; Dickman, 1996; Short and Turner, 2000)

What other factors, such as disease, that may have increased woylie vulnerability to predation (e.g. reduced the fitness, changed behaviour and/or morbidity) remains unresolved by this study alone. While this study clearly demonstrates that predation is a major factor associated with the woylie declines the evidence to date indicates that multiple factors are, in all likelihood, involved. Evidence that does not support predation as the primary cause of declines includes; i) little or no evidence or biological capacity for a pronounced increase in predator numbers capable of synchronous 25% – 95% annual decline rates in woylie numbers through much of the Upper Warren or elsewhere, ii) limited evidence of a clear catalyst that could precipitate an acute predator increase of this magnitude at this time and at so many sites, and iii) no decline in other sympatric prey species such as other 'critical weight range' medium-sized mammals (Burbidge and McKenzie, 1989) and large reptiles such as *Varanus, Egernia, and Trachydosaurus*. Prey switching (e.g. Krebs, 1985) by itself is not a likely explanation as to why only woylies have been targeted by a predator because woylie numbers have been reduced well below densities by which it would efficient for a predator to continue targeting woylies and secondly there is no evidence of other sympatric prey species being subject to prey switching either pre or post woylie declines. The only possible exception is the wambenger (*Phascogale tapoatafa*) which underwent a substantial decline in the forested areas of the Upper Warren region in 1995, however, the temporal link is weak and associative evidence indicates that these declines were related to food resources (Scarff, 1998; Rhind, 2002; Rhind and Bradley, 2002; Wayne unpublished data).

In summary, while predation is a *proximate* mortality factor, the *ultimate* factor(s) that have lead to this endpoint are likely to be non-predator related (i.e. it is most likely that predators are effectively exploiting a prey resource made susceptible by other factors).

4.3.4.4. Predation and scavenging

The review of available forensic evidence at the completion of the field program was a particularly useful exercise. As well as providing a training and active learning opportunity for the personnel involved, it helped to standardise evidence collection recording and interpretation and clarify the similarities and differences between the mortality cases. The similarity of the body remains between woylies identified as having been primarily predated/scavenged by the same species was particularly striking. The strength of these similarities increased the confidence of the interpretation of the evidence and provides the opportunity to develop better 'profiles' for the predators involved.

In the same way that is often not possible to distinguish between a predation and the scavenging of a moribund animal or recent death, particular care is required to discriminate between the first predator/scavenger and subsequent visitors to a carcass. The limitations in being able to do this need to be acknowledged and the use of associated forensic evidence needs to be considered with this in mind. For example, chuditch were identified by forensic odontology to be associated with 10 woylie bodies. While in all of these cases the balance of evidence was interpreted to indicate secondary scavenging, it cannot be unequivocally rejected that chuditch may have been the primary predator/scavenger.

4.3.4.5. Operational considerations

Survival monitoring

- Aerial surveillance was a particularly efficient (time, cost and personnel resources) and effective means of scanning the radio-signals of Upper Warren cohorts.
- Radio-collar issues were a significant concern for this study. A conservative approach with respect to injuries was adopted in consideration of the welfare of the woylies involved (the highest priority at all times), and as a result a large proportion of collars had to be removed to prevent further skin lesions. The collar also often provided a site for ectoparasites (especially ticks) to aggregate, which in themselves could become sites for infection. Careful consideration of the design and application and monitoring of radio-collars is required to minimize any potential welfare issues.
- The rapid detection and recovery of animal remains as soon after mortality is critical to the successful identification of the factors associated with death. Every hour after death

increases the loss and/or confounding of evidence as a result of decomposition and reduces the ability to identify proximate and ultimate causes of death. Mortality-sensitive collars set to trigger after no more than 2-3 hours of no movement, and daily monitoring as close to sunrise as possible are important considerations.

Forensics

- The forensics course and workshop met its objective to bring more science, rigor and skills to evidence collection and interpretation. Given its importance to this and other studies, further improvement would be readily achievable through further co-ordinated and collaborative work and training. A Corporate-level management of this would be optimal given its relevance and broader application within DEC (especially Nature Conservation and Science Divisions).
- The development of predator/scavenger 'profiles' would be a particularly valuable improvement given the fragmented nature of the existing evidence (i.e. a number of individuals each have a few bits of evidence that, when combined are substantially more valuable than the sum of the fragments in isolation). Predator/scavenger profiling would be well suited to a forensics student project (i.e. evidence collation and development of the behaviour, preferences and characteristic differences in the method of killing and consuming carcasses by difference predators).
- The removal of vital organs and tissues by predation/scavenging results in removal/alteration of evidence needed to determine whether underlying factors were the principal cause of death and/or increased the susceptibility to predation. The extent of evidence loss by this means has significantly compromised evidence collection in this study.
- The collection of associated evidence from DNA and odontology has been especially informative in this study. The value of pathology has been limited by the amount of useful material recovered with the body. Further improvements would be gained by a further development and refinement of these tools and the identification of other possible methods.
- Complication factors include; potential for multiple visitors (predators/scavengers), rate of decay and loss/disturbance of evidence over time; habitat factors – i.e. sandier and more open environments can provide more readily detectable sign such as distinctive foot prints associated with the body, signs of struggle (i.e. predation vs scavenging), etc.

4.3.5. Future work

- Analyses of the data and evidence remain to be completed, including; more sophisticated survivorship analyses (e.g. MARK), DNA evidence, trapping data to support evidence that emigration is not involved, etc.
- Karakamia radio-telemetry data associated with Andrew Hide's Honours research (Curtin University) will provide comparative survival data. Once the thesis is submitted, this will be available for comparison.
- Development of forensic profiles of common predators, particularly chuditch, fox, cat and raptors. This could be developed in collaboration with a forensics post-graduate student.
- Woylie 'search and rescue' forensic evidence has not yet been fully processed for evidence.

Operational matters

a. Improve DNA sampling and analytical techniques.
b. Improve collar design and application techniques to reduce adverse collar wear

Reduce the time from a mortality event until the forensic investigations is completed.

- Forensic training and development for personnel associated with any future work diagnosing woylie declines.

4.3.6. Conclusion

- Adult mortality is associated with woylie declines. The rate of mortality is higher in the declining population than the sites which had stable populations.
- The lack of predator data (activity and/or numbers) prior to this study means that it is not possible to examine the relationship and compare predators with earlier declines in the Upper Warren or elsewhere.
- Emigration is not involved in the loss of individuals from the populations undergoing decline.
- The cat was associated with most woylie mortalities. The fox has had a relatively minor association with woylie mortalities, which was equivalent to the level of mortalities associated with raptors.
- Chuditch has been commonly associated with woylie mortalities based on forensic odontology. Chuditch is more likely to be a secondary scavenger than a primary predator.
- Most of the predations involved almost complete consumption of the carcass, generally leaving little more than bones, skin and some muscle tissue (i.e. none of the major organs available for pathology). Therefore, identifying other possible factors associated with mortality have been substantially hampered.
- Predation is likely the *proximate* cause of mortality, the *ultimate* cause(s) of mortality (and woylie population declines) are likely to be something else other than predation, such as disease.

4.3.7. Acknowledgements

We are grateful for the contributions and assistance provided by countless individuals. In particular we thank the SES volunteers, community volunteers and Bunbury Cathedral Grammar students involved in the Woylie Search and Rescue exercise; presenters and contributors to the wildlife forensics course; project collaborators including Oliver Berry, Denice Higgins and Murdoch University Pathologists including Graeme Knowles, Phil Nicholls, Alexander McLachlan. Shane Raidal and Mandy O'Hara; DEC personnel based in Warren region that assisted with the collection and processing of forensic evidence in the field and laboratory. Colleagues associated with the mesopredator research program, particularly Neil Thomas, Nicky Marlow, Paul de Tores, Keith Morris and Dave Algar; and, the Manjimup Aeroclub, particularly the pilots Peter McGinty and Peter Davis.

4.3.8. References

Burbidge, A. A., and N. L. McKenzie. 1989. Patterns in the modern decline of Western Australia's vertebrate fauna: causes and conservation implications. Biological Conservation 50:143-198.

Christensen, P. E. S., and N. D. Burrows. 1994. Project Desert Dreaming: experimental reintroduction of mammals to the Gibson Desert, Western Australia. Pages 199-207 in M. Serena, editor. Reintroduction Biology of Australia and New Zealand Fauna. Surrey Beatty and Sons, Sydney.

Dickman, C. R. 1996. Overview of the impacts of feral cats on Australian native fauna. Australian Nature Conservation Agency, Canberra.

Krebs, C. J. 1985. Ecology: The Experimental Analysis of Distribution and Abundance. Harper Collins Publishers, New York.

Molsher, R., A. Newsome, and C. Dickman. 1999. Feeding ecology and population dynamics of the feral cat (*Felis catus*) in relation to the availability of prey in central-eastern New South Wales. Wildlife Research 26:593-607.

Rhind, S. G. 2002. Reproductive demographics among brush-tailed phascogales (*Phascogale tapoatafa*) in south-western Australia. Wildlife Research 29:247-257.

Rhind, S. G., and J. S. Bradley. 2002. The effect of drought on body size, growth and abundance of wild brush-tailed phascogales (*Phascogale tapoatafa*) in south-western Australia. Wildlife Research 29:235-245.

Scarff, F. R., S. G. Rhind, and J. S. Bradley. 1998. Diet and foraging behaviour of brush-tailed phascogales (*Phascogale tapoatafa*) in the jarrah forest of south-western Australia. Wildlife Research 25:511-526.

Short, J., and B. Turner. 2000. Reintroduction of the burrowing bettong *Bettongia lesueur* (Marsupialia: Potoroidae) to mainland Australia. Biological Conservation 96:185-196.

Wayne, A. F. 2006. Interim assessment of the evidence for a recent decline in woylie abundance in south-western Australia. Pages 1-28. CALM, Perth, Western Australia.

Wayne, A. F., I. Wilson, J. Northin, B. Barton, J. Gillard, K. Morris, P. Orell, and J. Richardson. 2006. Situation report and project proposal: identifying the cause(s) for the recent declines of woylies in south-western Australia. A report to the Department of Conservation and Land Management Corporate Executive. Pages 1-28. CALM, Perth, Western Australia.

4.4. Predators

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Abstract

Predator activity and fox control operations in the Upper Warren region were investigated in relation to their possible association with recent woylie declines. Predator activity surveys, using sandpads, were conducted at the five Upper Warren Population Comparison Study sites during the 12 months commencing August 2006. The surveys were conducted immediately prior to and after the quarterly fox-baiting events performed by DEC as part of the '*Western Shield*' program and Donnelly District conservation management.

Cat activity was least at sites with stable and high woylie abundance and greatest at Balban where woylies were currently declining, however, overall there was no statistically significant difference between sites. The preliminary evidence remains consistent with cats potentially having a role in the decline of woylies, however, it is premature and there is insufficient data to determine the strength or nature of this association.

Fox activity was significantly different between sites and generally increased overtime, particularly from February 2007. Fox activity did not differ significantly between pre and post fox-baiting and whether this is related to baiting effectiveness is uncertain due to limitations in the data and statistical power. Fox control activities have been highly variable in the Upper Warren region since 1996. Although generally within the *Western Shield* baiting framework, intervals in the fox control program have frequently occurred. More strategic spatial and temporal considerations of fox control, particularly in relation to fox biology, would be expected to substantially improve effectiveness with negligible impact on existing resources.

Methodological considerations of sandpad monitoring are also addressed. Sandpads across the full length of forest tracks are preferable to the 1 m^2 plots previously used in the region and passive sandpads were found sufficient for measuring fox and cat activity. The activity indices (AI) derived from the sandpads closely matched the capture rates for other fauna species.

Ongoing monitoring of predator activity and/or abundance would be extremely valuable and highly recommended for the Upper Warren and elsewhere where predator control is conducted and fauna conservation is considered a high priority.

4.4.1. Introduction

One of the multiple competing hypotheses (*sensu* Peery *et al.,* 2004) of the Woylie Conservation Research Project is the possible role of predators in the recent woylie declines. Likely predators include the introduced fox and cat, and native predators, such as the chuditch and the wedge-tailed eagle. The likely role of predation is supported by the evidence that the fox was principally responsible for historical declines of woylies across its former range throughout southern and central Australia (Burbidge and McKenzie, 1989; Start *et al*., 1995) and the spectacular recovery of the species in the presence of fox control (e.g. Start *et al*., 1998).

To assess potential associations between predators and woylies sandpad surveys were conducted at the five Upper Warren Population Comparison Study (PCS) sites.

The primary aims of the PCS predator investigation were to;

1. Measure predator activity at the Upper Warren PCS sites (fox, cat and chuditch)

2. Collate the history of predator control operations in the Upper Warren region
3. Assess the timing of current fox control activities in relation to significant rainfall events (i.e. assessment of potential baiting effectiveness)
4. Relate predator activity to the fox baiting program (i.e. compare predator activity pre and post baiting), and
5. Relate all of this evidence to the patterns of recent woylie declines (spatial and temporal)

This research also provided an opportunity to consider aspects of the sandpad methodology used to examine predators for the purposes of improving its use in possible future applications. Therefore, secondary, (methodological) aims of this research included;

1. Assessment of sandpad design – differences in the detection of predators on passive versus active sandpads (i.e. the use of selected audio and olfactory lures)
2. Development of improved sandpad survey methods (opportunistic)
3. Use of sandpads as an indicator of activity of native fauna (e.g. chuditch, woylie, koomal, and species not readily trapped such as large macropods).

This report predominantly focuses on the introduced fox and cat given their higher likelihood of being involved in recent woylie declines compared with the native predators, which are themselves conservation-listed species. Elements of the predator investigation not addressed in this report include; i) predator scats (predominantly chuditch) collected for dietary analysis, ii) chuditch population data from cage trapping activities, and iii) wedge-tailed eagle records of active nest sites and sightings. This data will be used for possible future investigations. Based on the existing records of opportunistic sightings it is considered unlikely that the wedge-tailed eagle has increased sufficiently in density to be primarily responsible for the substantial and rapid woylie declines within the Upper Warren region and so have not been addressed in detail in this report.

4.4.2. Methods

4.4.2.1. Upper Warren site descriptions and history

The predator component of the project involved all of the Population Comparison Study (PCS) sites – Keninup, Balban, Warrup, Boyicup, Winnejup and Karakamia Wildlife Sanctuary.

Fox baiting history

Historically, predator control within the Upper Warren region was predominantly done for agricultural and stock protection purposes, principally by local farmers and typically in an uncoordinated and sporadic manner using a range of methods. Opportunistic and targeted shooting (and sometimes baiting) of foxes and cats by farmers continues and remains variable spatially, temporally and methodologically. Although this is difficult to quantify, it should be considered as another factor that may relate to observed predator activity levels.

Fox-baiting for the conservation of native fauna and research occurred in two areas of Perup Nature Reserve between 1977 and 1990 (Burrows and Christensen, 2002). Some areas, particularly in the northern part of the Upper Warren, also were occasionally ground-baited to reduce wild dogs (*Canis familiaris*) and foxes and protect nearby livestock between 1986 and 1992. Between 1992 and 1998, a strategy was introduced to ground-bait selected areas for conservation purposes, the selection being based partly on the existing diversity and abundance of native medium-sized mammals (I. Wilson, unpublished data). Extensive aerial fox-baiting (four baitings per year) within most of the publicly-managed forests of the Upper Warren began in 1996 as part of the '*Western Shield*' conservation programme (Department of Conservation and Land Management, 2000; Orell, 2004).

As part of the current *Western Shield* program the area encompassing the PCS sites is aerially baited four times per year. Ground-baiting is conducted in conjunction with the aerial baiting program but concentrates on the interface between private property and DEC-managed estate.

PCS sites in relation to agricultural activities

The potential impact of DEC-managed predator control activities on each of the PCS sites differs based on proximity, interface and extent of private property within the area. The location of each sandpad array and the relevance of each of these variables can be seen in Figure 4.4.1. when overlaid with the aerial and ground baited zones.

Keninup (Figure 4.4.2.) has the greatest extent of forest edge to private property as it is on the northern extent of the aerially baited zone and is adjacent to unbaited land to the north, east and west. In comparison Balban (Figure 4.4.3.) has the least cleared agricultural land to forest ratio but there is a large corridor leading into the southern end of Balban that is not aerially baited. Unlike both Keninup and Balban there are no sandpads greater than 500 m away from the aerially baited zone within Warrup (Figure 4.4.4.), Boyicup (Figure 4.4.5.) or Winnejup (Figure 4.4.6.). The Warrup array is contained the most within the aerial bait zone, and has only a small pocket around private plantations excluded.

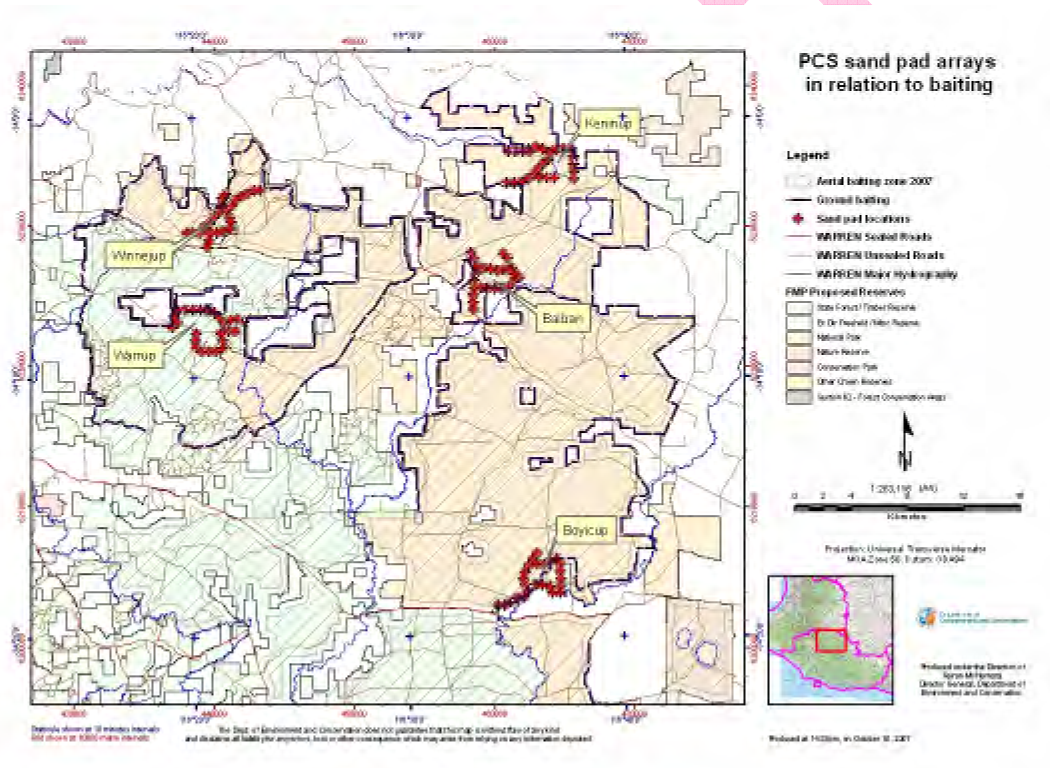


Figure 4.4.1. Overview of the sandpad arrays within the PCS sites in relation to aerial and ground-baited zones.

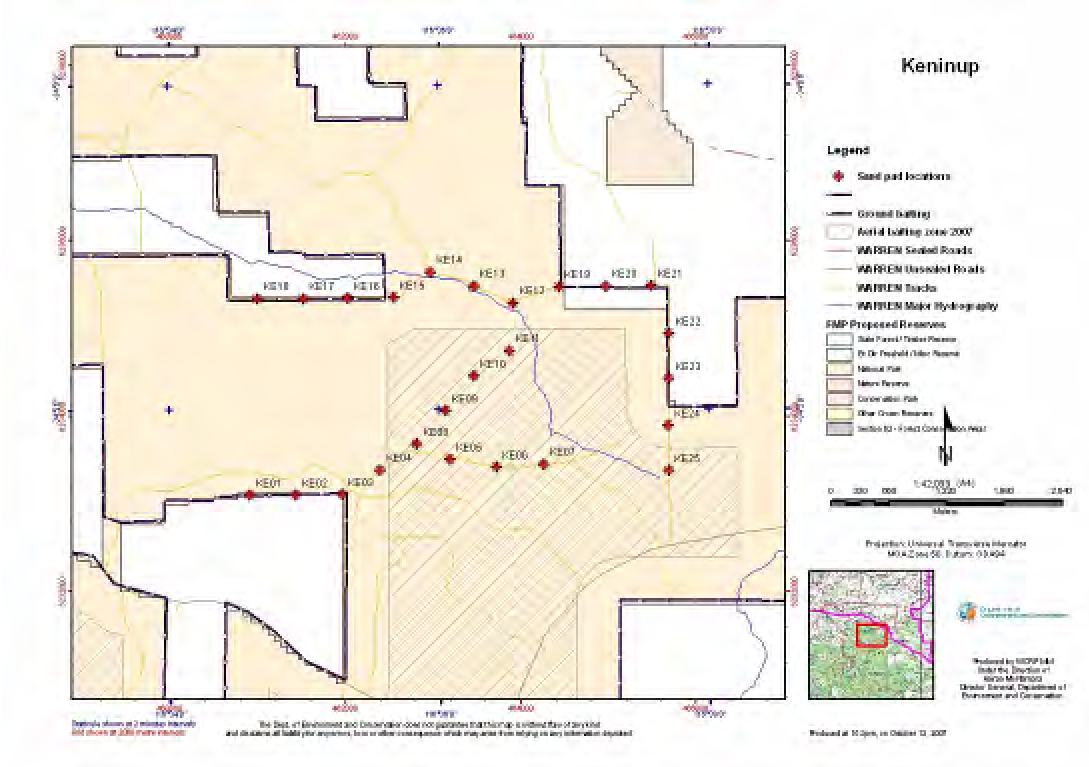


Figure 4.4.2. Keninup sandpad array.

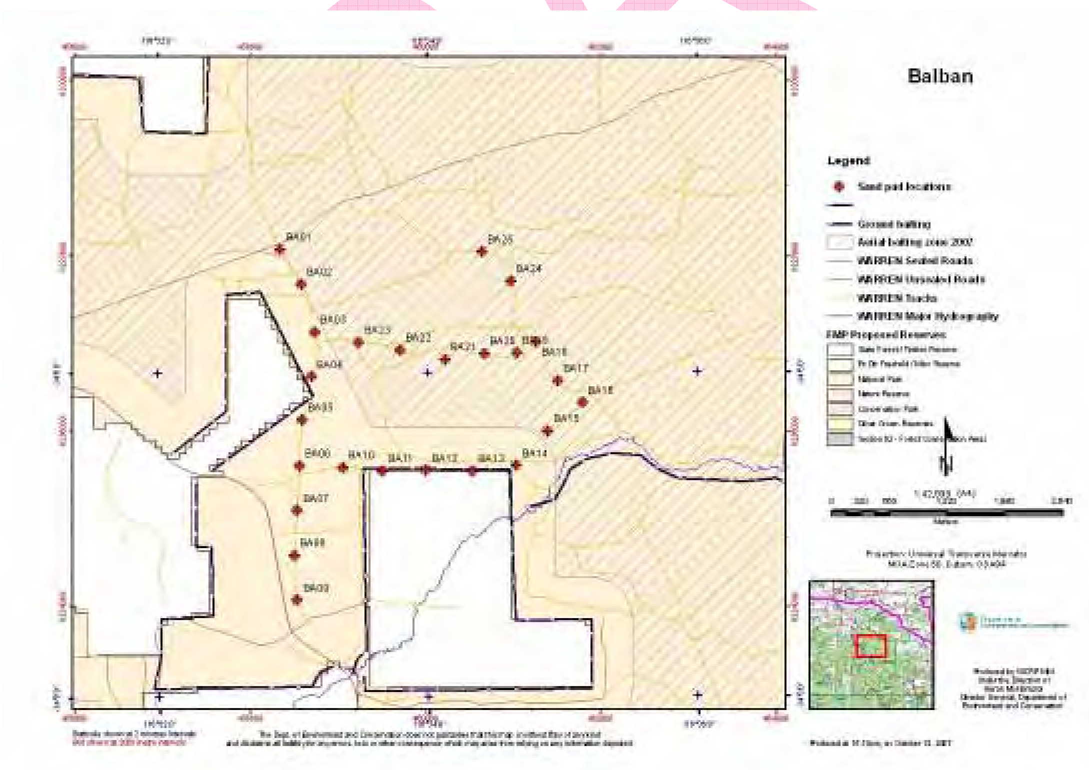


Figure 4.4.3. Balban sandpad array.

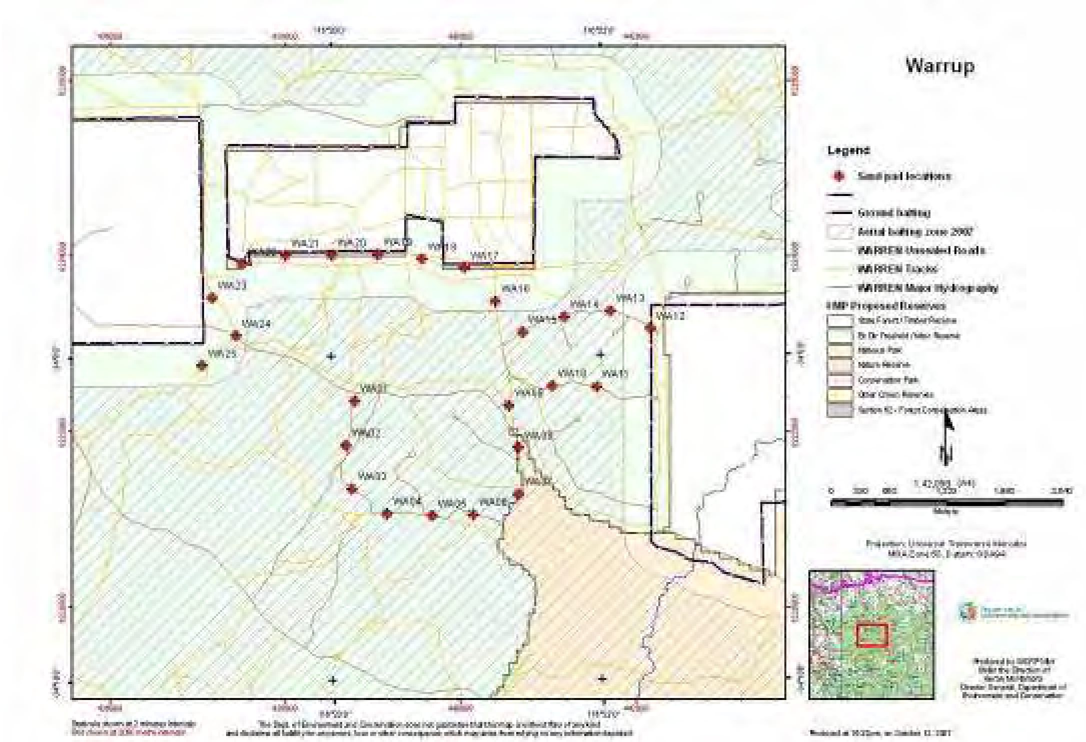


Figure 4.4.4. Warrup sandpad array.

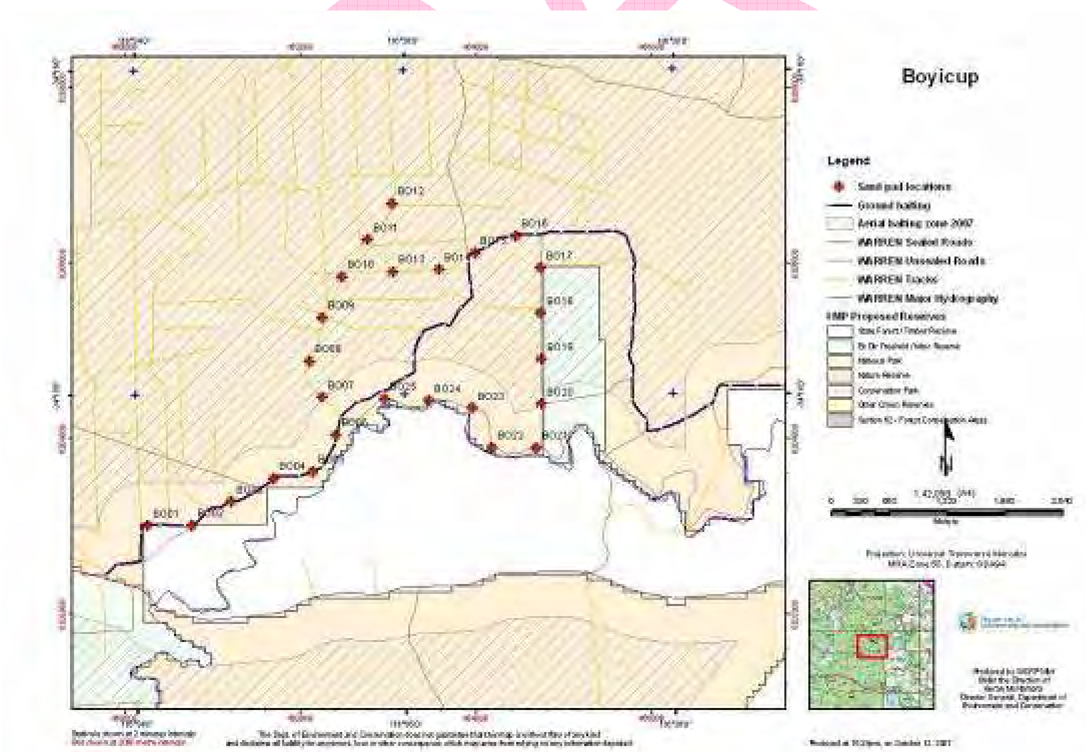


Figure 4.4.5. Boyicup sandpad array.

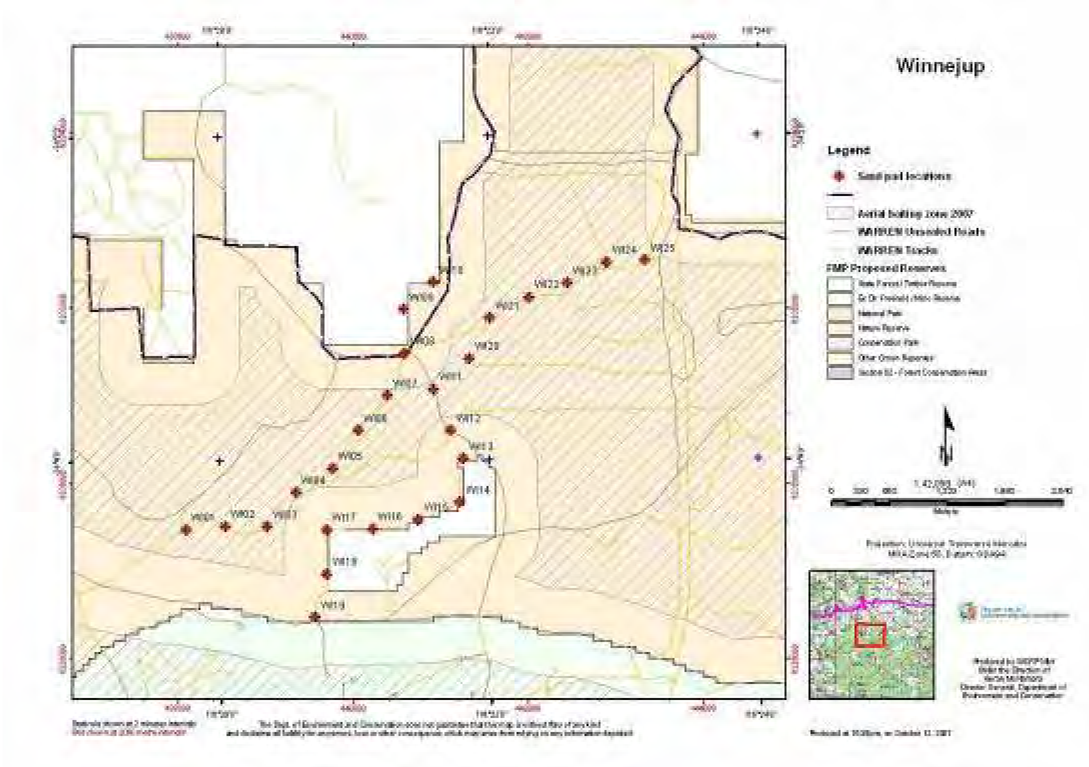


Figure 4.4.6. Winnejup sandpad array.

4.4.2.2. Karakamia

Karakamia Wildlife Sanctuary (managed by AWC) is surrounded by a 'predator-proof' fence and considered free of introduced predators. Predator surveillance is continual and control methods are only used when there is an incursion (Schmitz and Copley, 1997). Monitoring involves checking fence condition by quad bike once a week and then a close check by walking the fence once every 1-2 months. The functionality of the electric fence and gate are checked daily. A series of 14 sandpads on the inside of the fence line and one in the core are also checked opportunistically. General signs of predator incursion are continually monitored, including predator scats particularly along the boundary fences, and visual surveillance (Trish Gardner, pers. comm.).

In the last 15 years there have been at least three fox incursions. One of these was as a result of fence damage caused by a bushfire in 1996. Another was due to gate malfunction. These foxes were removed by 1080 baiting, utilising the sandpads. One cat was enclosed within the fence during the addition of a parcel of land to Karakamia and was target-trapped for removal (Trish Gardner, pers. comm.).

Natural predators do exist within Karakamia. These include a pair of breeding wedge-tailed eagles (Cherriman, 2007). Carpet pythons have been opportunistically observed with fresh woylie carcasses and have consumed radio-collared animals (Trish Gardner, pers. comm.). Chuditch have been caught during trapping surveys (Jacqui Richards, pers. comm.), although chuditch have not been released into the reserve (Trish Gardner, pers. comm.).

Given that Karakamia is effectively predator free, the Karakamia woylie population is not going to be addressed any further in this report in relation to predators.

4.4.2.3. Survey (Sandpad) design

Sandpad array

For each Upper Warren PCS site an array of 25 sandpads, spaced no closer than 500 m apart, were distributed along existing tracks and roads. Attempts were made to use tracks infrequently used by vehicle traffic (i.e. minimise pad disturbance) and arranged as compactly as practical to provide site-specific data. Consideration was also given to achieving a representative balance between forest edge and forest away from agricultural land.

Survey timing

The timing of surveys were directly associated with the quarterly fox-baiting events, although the exact timing of these events were not precisely known in advance (i.e. dependent on weather, availability of contractors, baits, etc). The objective was to conduct the pre baiting surveys as close to the anticipated baiting event as possible, and within a maximum of four weeks. Post-bait survey sessions were aimed to start at least 10 days and less than four weeks after the completion of a baiting operation. The surveys were conducted concurrently at each site, with pre-bait sessions in August and December 2006, February and June 2007 and post-bait surveys in October 2006, January, April, and July 2007.

Sandpad dimensions and characteristics

The sandpads were at least one metre wide x 50-100 mm deep across the entire track from ‘batter to batter’. Depending on the width of the track this varied from three to five metres.

The minimisation of the risk of introducing and/or spreading plant dieback from *Phytophthora* infection was a particularly high priority for the construction and operation of the sandpads (i.e. best-practice observed). The sand used on the pads was sourced locally from a sand pit near Manjimup that was managed under strict hygiene conditions, and was considered *Phytophthora* free (confirmed by soil analysis - 10 samples tested negative by Vegetation Health Service, DEC Science Division, 17 Dick Perry Ave, Kensington, WA 6152).

The sandpads were constructed using either a kanga or backhoe to dig a shallow trench, which when filled with sand was approximately flush to the surrounding ground surface. Gypsum was added to all sandpads during the December survey and mixed through with a mechanical cultivator, to improve sandpad quality.

Sandpad preparation

Refer to the WCRP Operations Handbook (Volume 3) for a detailed description of the operational protocols and a materials list. In summary, the preparation of the sandpad involved clearing debris, ‘harrowing’, and sweeping to achieve a flat, consistently light and friable substrate suitable for reading sign from vertebrates. Adding moisture to very dry sandpads was not routinely done given that superficial application resulted in an undesirable surface crust and more complete sandpad moisturising was logistically impractical.

Alternate sandpads were consistently passive (no lure) and active (lure) along each sandpad array (12 and 13 pads respectively). The active sandpads had both a scent and auditory lure. A capful of fish oil (Bait Mate Tuna Oil©, 6/61 Buckingham Drive, Wangara WA 6065) was placed in the centre of the sandpad between the wheel ruts and replenished every day of the survey. A FAP (Felid Attracting Phonic) was tethered at one end of the sandpad in the same place each survey session.

Data protocols and collection

- Refer to the WCRP Operations Handbook (Volume 3) for a detailed description of the data recording protocols and ‘data sheet’.
- The key species recorded from the sandpads included the cat, fox, chuditch, woylie, koomal and macropod. All other fauna including quenda, birds, reptiles, etc were also recorded in an “other” field.

- A print identification confidence rating was applied to each record (1-certain, 2-probable, 3-possible).
- Pad condition was described, e.g. ok, washed out by rain, etc.
- Weather conditions were recorded particularly in reference to local rain events given the variability between sites and during the day.
- Description of predator activity included the size, direction and location of foot prints on the sandpad, and number of sets of prints. Also whether the predator visited the FAP and / or olfactory lure.
- Predator scats found on the sandpads were collected, labelled and dried (3-4 days at 35 degrees centigrade) and stored at room temperature. These remain available for DNA confirmation and diet analysis.
- Photographic records were taken for reference purposes and for unusual or difficult to identify prints.

4.4.2.4. Measures of predator activity

Predator activity index is a relative measure of encounter rate that can be used as a simple estimation of the probability (or risk) of a woylie encountering a predator, notwithstanding the assumptions required to do so (i.e. activity, behaviour, and interactions between and within prey and predator species). This is likely to be more useful than estimates of predator density/numbers derived from sandpad encounter data given the additional assumptions and limitations associated with converting activity-based data into population estimates.

Allen's activity index

Originally developed for dingoes, the Allen's index (Allen *et al.,* 1996) is applicable for other species. In summary, the Activity Index (AI) for a species (per site/session) is the average of the daily calculation of the total number of sandpads with confidently-identified prints divided by the total number of available (readable) sandpads. Only records with a confidence rating of one were included in analysis. A variant (EPA / QLD PWS, 2007) of the Allen Activity Index was used in this study. The basis of the variation to Allen's AI is the decision set for determining the available pads as the denominator and the number of sets of prints for a species to be used as the numerator.

A more robust AI can be calculated if all sandpads that don't have the potential to leave clear prints are removed from the AI calculation. In this study, field comments on sandpad condition were classified as an estimate of decipherability (1 = good - ok; 2 = moderate, 3 = poor). However, consistency and subjectivity issues associated with this approach were problematic, and so, was not used in the final analysis. Instead, survey days where sites were affected by heavy overnight rain were removed from the analysis, and individual sandpads were only removed if disturbed by vehicles and stock, irrespective of the extent of disturbance.

The presence/absence of confidently-identified prints on a sandpad was used in this study, as opposed to Allen *et al.,* (1996), by which the number of sets of prints was used. In so doing, this study avoids the need to consider assumptions of independence between sets of prints.

Changes in activity indices for each species were estimated using a generalized linear model. Any temporal trend in activity was accounted for by using a quartic polynomial (i.e. effects were fitted for the first four powers of time) as a covariate, before testing for site differences and changes pre- and post-baiting (treatment effects), and their interaction. Models were fitted using SAS statistical software (SAS, 2006).

4.4.3. Results

4.4.3.1. Fox baiting history

Aerial baiting

Table 4.4.1 summarises the timing of all aerial baiting sessions in relation to the amount of rain for the Upper Warren region, since the commencement of *Western Shield*. The timing of baiting has not been consistent over time. The number of baiting sessions per year has ranged from 3 - 6 (average=4).

The interval between successive aerial baiting sessions ranged from 40 to 189 days (average=90.9, SE=4.22). Winter baiting events have been more frequently associated with substantial rain than not – 54% of baiting events in June/July were associated with >40 mm rain within two weeks after baiting. A total of 20.9% of all aerial baiting events were associated with >40 mm rain within two weeks after baiting. If these high rainfall events were considered likely to be ineffective at fox control, the interval between successive effective baiting events has been up to nine months (2003).

Ground baiting

The Upper Warren region is divided into two cells for ground baiting, Kingston and Perup. Hand baiting duration for the Kingston cell ranged from 1 - 13 days (average=2.2, SE=0.28) and for the Perup cell, 2 - 13 days (average=4.9, SE=0.50). Aerial and ground baiting have not been conducted simultaneously. The lag between aerial and hand baiting for the Kingston cell ranged from -15 - 64 days (average=6.9, SE=2.44). The lag for the Perup cell ranged from -10 - 42 days (average=13.7, SE=2.11) (Table 4.4.2.). On nine occasions either the Perup or Kingston cell was not ground baited at all following aerial baiting. The period between successive Kingston ground baiting sessions ranged from 25 - 279 days (average=98.2, SE=6.48) and for Perup the range was from 52 - 294 days (average=100.4, SE=6.87). The largest intervals in the baiting program generally occurred over the summer months from December to April, coinciding with the dispersal period of young foxes (Saunders *et al.,* 1995; de Tores, 1999; Thomson *et al*., 2000).

Table 4.4.1. Aerial fox baiting history in the Upper Warren in relation to rain.

Year / month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1996											X	
1997			X			X				X		
1998				X		X				X		
1999	X		X		X		X			X		X
2000				X			X				X	X
2001				X		X				X		X
2002			X				X		X			X
2003			X			X			X			X
2004			X			X			X			X
2005				X			X		X			X
2006			X				X		X			X
2007			X			X						

X < 30 mm rainfall in 14 days post baiting = assumed effective

X = 30 mm - 40 mm rainfall in 14 days post baiting = possibly effective

X > 40 mm rainfall in 14 days post baiting = potentially ineffective

Table 4.4.2. Ground baiting history for the Kingston and Perup cells of the Upper Warren region including number of lag days between aerial and ground baiting sessions.

		Month											
Year	**Site**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
1997	K	-12		*	16		*	19			*-8		
	P	7		*	30		*	22			*-3		
1998	K				*	34	-7	*			*3		-12
	P				*	19		*5			*17		
1999	K	*		*	11	*		*-6			*	20	*
	P	*	36	*	22	*		*2			*	32	*
2000	K				*-7			*-15				*17	*
	P				*	8		*-8				*	*
2001	K				*	11	*		64		*4		*
	P				*	17	*			-10	*		*
2002	K			*				*-2		*7			*0
	P			*	11			*5		*	14		*-8
2003	K			*	24		*-1			*8			*6
	P			*	27		*8			*	23		*14
2004	K			*	20		*14			*9			*
	P			*	28		*21			*16			*
2005	K	21			*15			*-9		*9			*5
	P	23			*24			*-1		*	42		*
2006	K			*6			-15	*		*7			*0
	P	11		*	25			*-5		*	13		*2
2007	K			*9			*-1						
	P			*13			*4						

K=Kingston cell, P=Perup cell

*aerial baiting event

No. of days = difference between last day of aerial baiting and last day of ground baiting.

4.4.3.2. Species detected from sandpads – raw data

The incidences of cat and fox records were low relative to chuditch (except Boyicup) and native 'prey' (Figure 4.4.7.). The koomal was the most commonly recorded species (total of 1495 records) and had the highest representation at all sites except Keninup, where the woylie was the most common. Quenda records were not common but were particularly low at Balban and Boyicup (n=1).

Other non-mammalian natives and other introduced mammals were also occasionally detected on the sandpads (Table 4.4.3.). Varanids were the most commonly recorded reptiles, which also included some bobtail and skink-sized prints. Raven and magpie sized birds were the most common birds detected, as well as some cockatoos and smaller perching birds. Rabbit presence was generally localised to private property boundaries.

The dog prints detected at Winnejup and Keninup were immediately adjacent to agricultural land and were usually associated with human activity (i.e. neighbouring landholder dogs). There was no evidence of associated human activity for the dog prints detected at Balban or Boyicup. Dog prints were evident at Boyicup during the August 2006 and April, June, July 2007 survey sessions. Overall, dog activity and potential threat to the woylie was minimal and as a result is not discussed further in this report.

Other rare and interesting prints identified on the sandpads included one record each of wambenger and numbat, at Keninup and Balban, echidna at Balban (n=1), and dunnart at Boyicup (n=1).

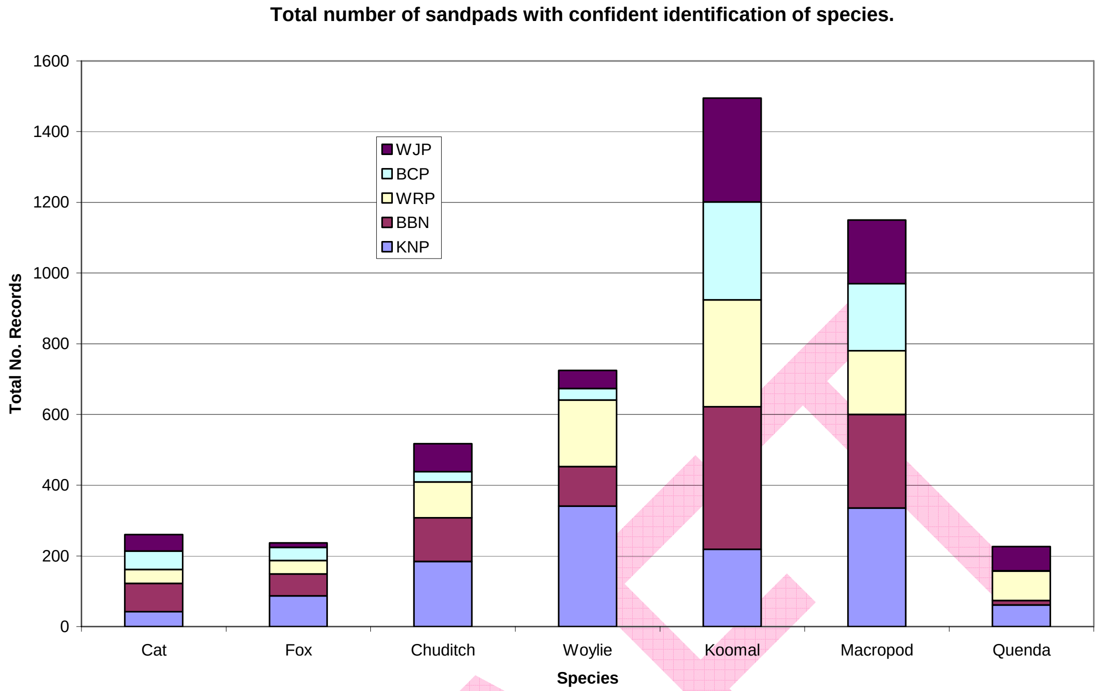


Figure 4.4.7. Representation of the key species recorded on the sandpads.

Table 4.4.3. Total number of records of other species identified on the sandpads.

Site	Native			Introduced		
	Emu	Bird (not emu)	Reptile	Rabbit	Dog	Sheep
Keninup	32	174	39	33	3	1
Balban	4	86	33	5	3	4
Warrup	10	52	22	13	0	0
Boyicup	18	72	41	5	8	0
Winnejup	15	80	52	19	5	6
Total	**79**	**464**	**187**	**75**	**19**	**11**

4.4.3.3. Predator activity from sandpads

Suitable sandpads – AI denominator development

Exclusion of days at sites where heavy rain affected sandpads resulted in the removal of 20% of the sandpad data (Table 4.4.5.). Heavy rain impacts on sandpads during the October '06 and Feb '07 were especially disruptive. As a consequence, the October '06 AI results for all sites and the February '07 results for Boyicup and Warrup were based on limited data. Additional surveys at Balban and Keninup were conducted in the week following the Feb '07 session to partially compensate for the impacts of the heavy rain. As a result, the four survey days at Balban and Keninup in the February '07 survey were not consecutive. Additional compensatory surveys at other sites in Feb '07 were not conducted due to human resource limitations.

A total of 189 sandpads were disturbed by vehicles (motorbikes, passenger vehicles, tractors, etc) and removed from the analysis (approximately 5% of the sandpads otherwise available for the AI calculations; Table 4.4.5.). Keninup had the greatest disturbance from vehicles, followed by Warrup and Winnejup. Most (61%) of the vehicle incursions at Keninup occurred on the eastern boundary sandpads (#20 to #25).

Of the 3579 sandpad records used in the analysis, 154 were from sandpads rated as either moderate or poor condition (i.e. little influence on the AI calculations). The condition of sandpads on any one day varied considerably between sites as a result of substantial site differences in weather (especially rainfall and evaporation), road condition, aspect, etc. For example, Winnejup sandpads were the most affected by rain and hence an extra three days were removed from AI calculations compared to the other sites (Table 4.4.4.).

Footprints – AI numerator development

More cats had a lower print confidence (i.e. less than 1=confident) rating than foxes (10.8% and 4.6% respectively) - these instances were filtered out prior to analysis. Reasons for these differences include, cat prints were more difficult to decipher than fox on sand not of perfect quality, and differences in the weight and the behaviour of the species on the sandpad.

Using the presence/ absence of prints on the pads as the numerator in the AI calculation was considered robust and more reliable than an estimate of number of individuals per sandpad. Nonetheless, the alternative approach would not have ultimately affected the data significantly: there were only six cat and 18 fox cases where sandpads were found to have had probable two or more individuals present on any one pad (i.e. two or more sets of prints in the same direction).

Table 4.4.4. Sandpad survey session details for the Upper Warren PCS sites.

Session	Fox bait treatment	Aerial baiting date	Ground-baiting date	Session start date	Site	No. survey nights	No. nights heavy rain	Total survey nights per session
Aug06	Pre			22/8/2006	ALL	9	2	35
Oct06	Post	20/09/2006	29/09/2007	10/10/2006	ALL	4	2	10
Dec06	Pre			5/12/2006	ALL	4	0	20
Jan07	Post	19/12/2006	20/12/2006	16/1/2007	ALL	4	0	20
Feb07	Pre			27/2/2007	KNP	6	2	
					BBN	6	2	
					WRP	4	2	
					BCP	4	2	
					WJP	4	4	12
Apr07	Post	13/03/2007	20/03/2007	3/4/2007	ALL	4	0	20
Jun07	Pre			12/6/2007	ALL	4	1	15
Jul07	Post	29/06/2006	29/06/2007	10/7/2007	ALL	4	0	20
Total						189	37	152

Table 4.4.5. Total number of sandpads available for calculation of the activity index (AI).

Site	No. sandpads disturbed by heavy rain	No. sandpads disturbed by vehicles*	No. sandpads disturbed by stock*	Total no. available sandpads	% of the raw total
Keninup	175	75	0	725	74.4
Balban	175	6	4	790	81.0
Warrup	175	47	0	703	76.0
Boyicup	175	5	0	745	80.5
Winnejup	250	56	3	616	66.6
Total	**950**	**189**	**7**	**3579**	**75.7**

*Mutually exclusive of number sandpads disturbed by heavy rain.

Cat activity indices

Cats were detected at all sites during all sessions except two, one at Boyicup April 2006 and Winnejup January 2007 (Figure 4.4.8.). Proportionally more cat records were removed from Keninup than other sites due to disturbance, one case due to vehicle and 12 due to heavy rain (Table 4.4.6.). Balban then had the greatest number removed due to rain.

The overall average cat AI was highest at Balban, followed by Boyicup, Winnejup, Warrup and Keninup. However, there was no significant difference in activity between sites (p=0.3927, df=4). Cat activity differed significantly over time (p=0.0311, df=4) with a trend for increasing cat activity over time (Figure 4.4.9.). Very little cat activity was observed at Winnejup until April 2007 after which it increased markedly for the remainder of the study (Figure 4.4.8.).

There was no significant difference in the activity index between pre and post baiting (p=0.1534, df=1) (Table 4.4.12.) as would be expected.

Table 4.4.6. Summary of cat print records and activity indices from the sandpad surveys at the Upper Warren PCS sites.

Site	No. of +ve records	% of the raw total	Activity Index	SE
Keninup	29	69.0	0.041	0.009
Balban	61	76.3	0.075	0.013
Warrup	34	85.0	0.044	0.009
Boyicup	44	84.6	0.060	0.015
Winnejup	39	84.8	0.069	0.028
Total	**207**	**79.6**		

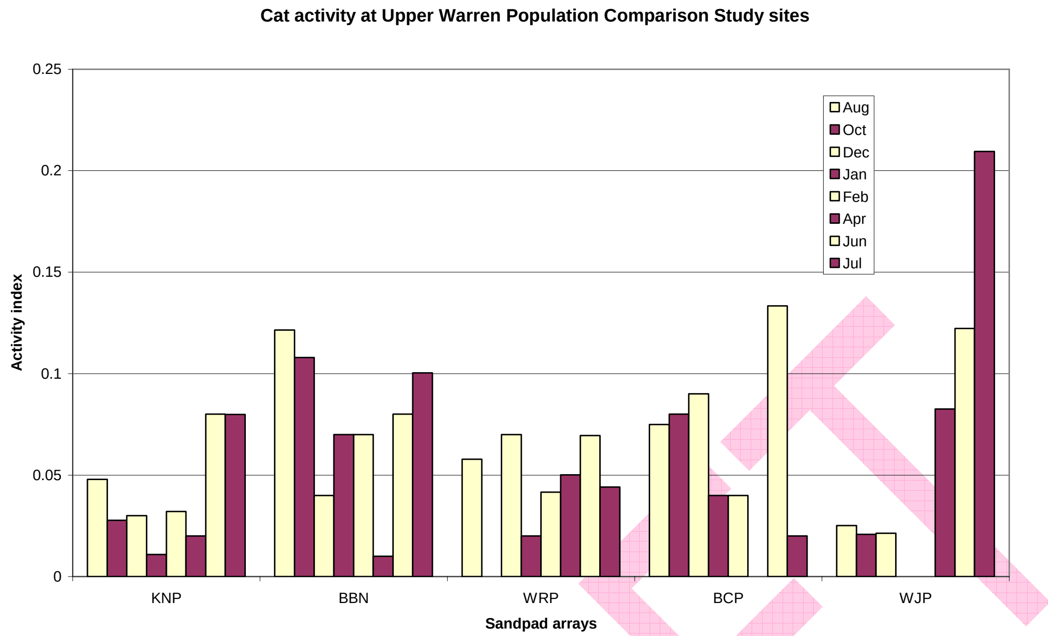


Figure 4.4.8. Cat activity index for each session at all Upper Warren PCS sites.

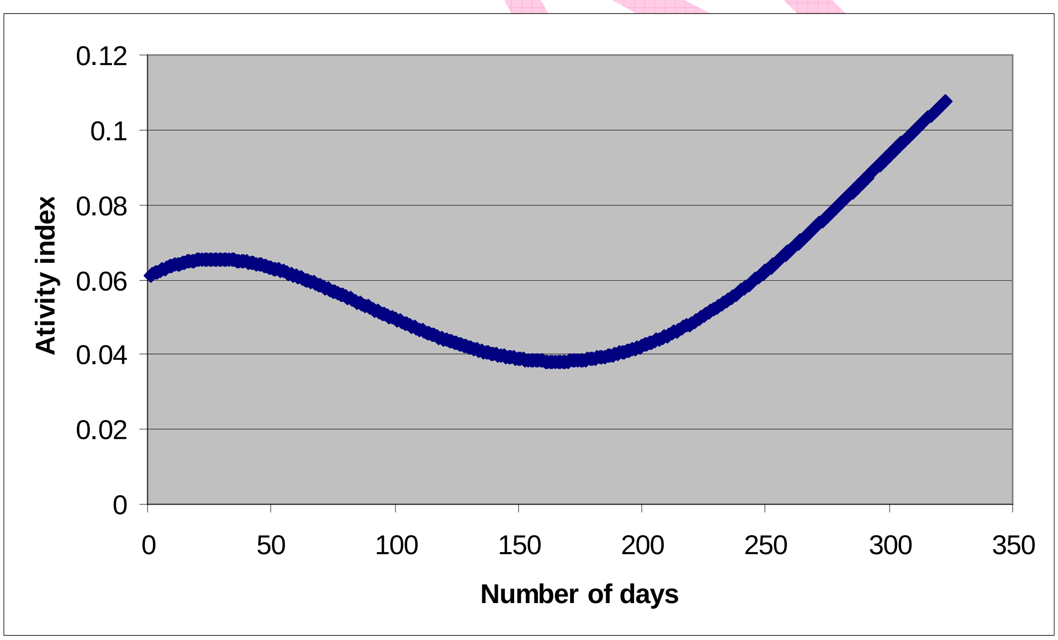


Figure 4.4.9. Temporal differences in the cat activity index for Upper Warren PCS sites.

Fox activity indices

Presence of fox activity was less consistent than cat activity, with a total of eight incidences where foxes were not recorded at a site during a session (Figure 4.4.10.). Of these incidences, four were during the October 2006 session. Fox activity at Keninup increased markedly in February 2007 and remained high through to the end of the study in July 2007. There was a similar trend at Balban. A large increase was also observed at Boyicup in July 2007 (Figure 4.4.10.). Fox activity was consistently low at Winnejup throughout the survey sessions.

There were only three cases of subadult size fox prints being recorded: Keninup 18/01/07 and 3/4/07; Balban 19/01/07.

There was a marginally significant difference in the fox AI between sites ($p=0.0593$, $df=4$), with activity being the highest at Keninup and least at Winnejup. The variance of session AI's were higher than for cat. Similarly, there was a greater fluctuation in the AI within sites between sessions (Table 4.4.7.). There was a significant difference in activity over time ($p<0.05$, $df=4$) with a general increase in fox activity (Figure 4.4.11.). There was however, no significant difference in the overall activity index between pre and post baiting ($p=0.3581$, $df=1$) (Table 4.4.12.).

Table 4.4.7. Summary of fox activity.

Site	No. +ve records	% of the raw total	Activity Index	SE
Keninup	68	78.2	0.095	0.027
Balban	58	93.5	0.075	0.025
Warrup	35	92.1	0.050	0.015
Boyicup	34	91.9	0.041	0.025
Winnejup	6	46.2	0.011	0.004
Total	**201**	**84.8**		

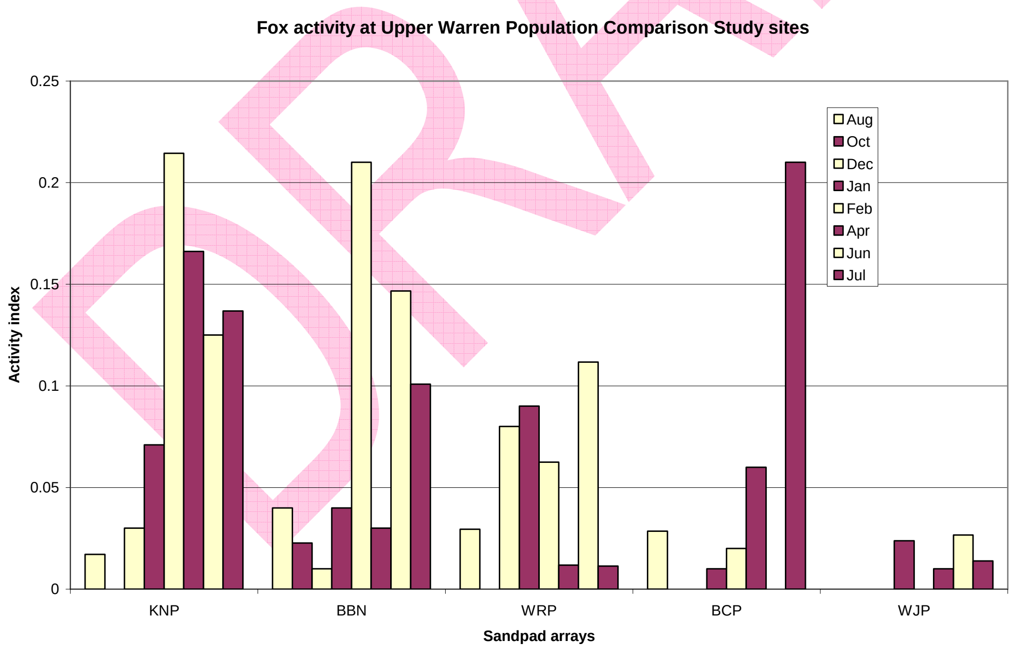


Figure 4.4.10. Fox activity index for each session at all Upper Warren PCS sites.

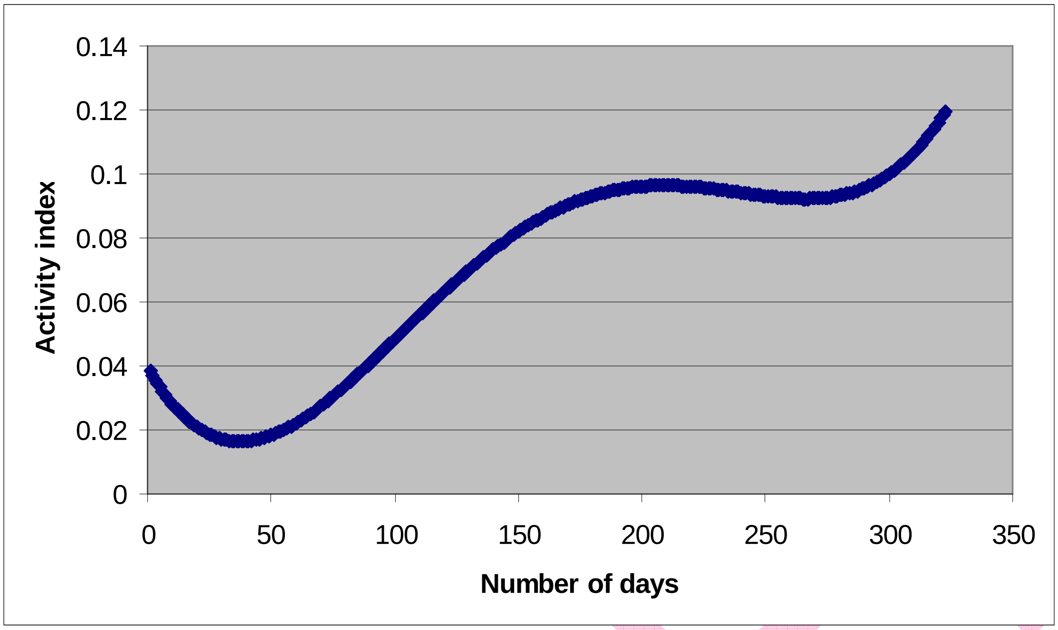


Figure 4.4.11. Temporal differences in the fox activity index for Upper Warren PCS sites.

Chuditch activity indices

Keninup overall had the greatest chuditch activity, followed by Balban, there was then a drop to Warrup and Winnejup and Boyicup had the lowest index of activity. This spatial difference was highly significant (p=<0.0001, df=4) with Keninup being higher and Boyicup lower than all other sites (Figure 4.4.12. and Table 4.4.8.).

There was an overall significant difference in chuditch activity for PCS sites over time (p<0.05, df=4). The activity fluctuated over time with a peak around May using a Type I covariate model (Figure 4.4.13.). There was no overall significant difference in activity between pre and post baiting (p=0.2786, df=1) (Table 4.4.12.).

Table 4.4.8. Summary of chuditch activity.

Site	No. +ve records	% of the raw total	Activity Index	SE
Keninup	151	82.1	0.218	0.034
Balban	114	91.9	0.146	0.022
Warrup	79	78.2	0.111	0.035
Boyicup	23	79.3	0.035	0.010
Winnejup	62	78.5	0.108	0.028
Total	**429**	**83.0**		

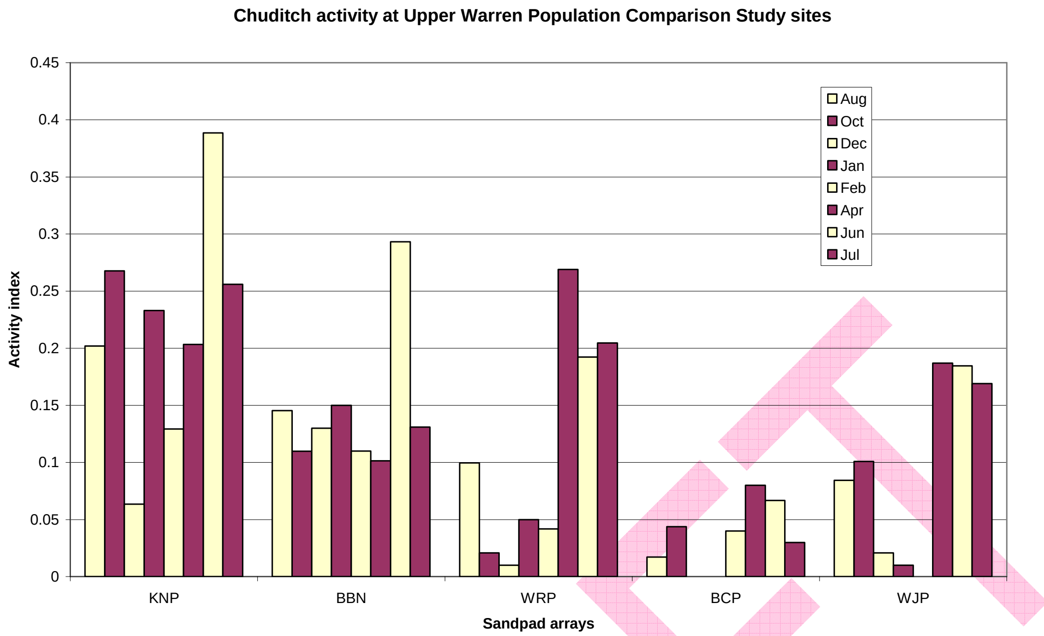


Figure 4.4.12. Chuditch activity index for each session at all Upper Warren PCS sites.

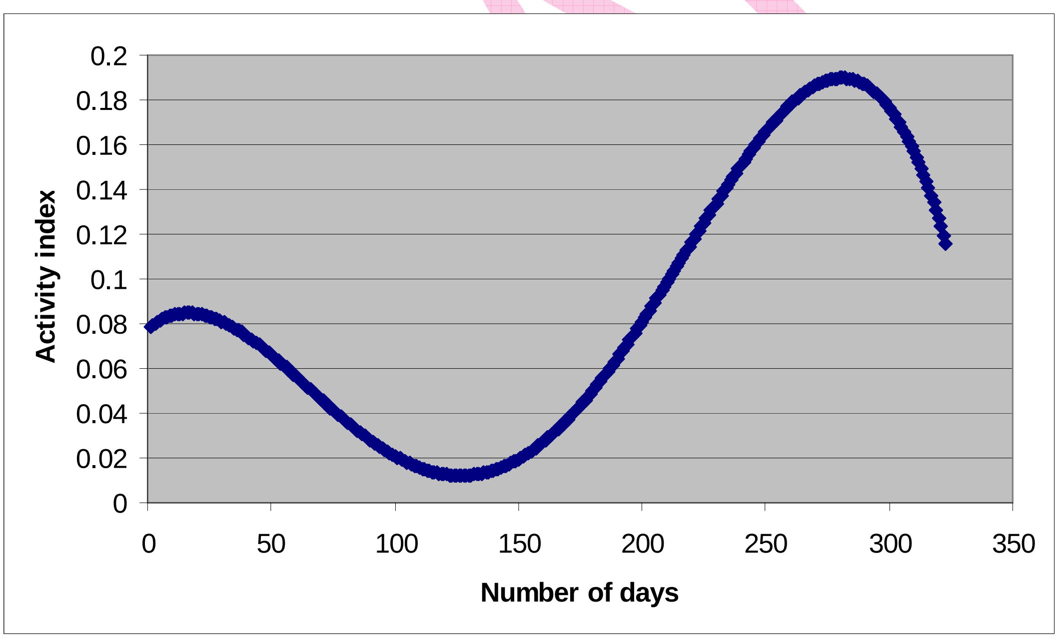


Figure 4.4.13. Temporal differences in the chuditch activity index for Upper Warren PCS sites.

Woylie activity indices

A highly significant difference between sites existed for woylie activity ($p=<0.0001$, $df=4$), particularly due to a significantly higher activity observed at Keninup than all other sites. Warrup activity was then significantly higher than the next highest site Balban. Winnejup then Boyicup had the lowest activity index (Figure 4.4.14.). In the case of Boyicup this equated to, on average, less than one woylie record per day (Table 4.4.9.).

There was a significant change in activity over time ($p<0.05$, $df=4$). Overall the trend was for a slight decline in activity (Figure 4.4.15.), due predominantly to the declines observed at Balban. There was some decrease in the AI at Keninup but this then increased again. Winnejup and Boyicup fluctuated with very low woylie records (Figure 4.4.14.).

Table 4.4.9. Summary of woylie activity.

Site	No. +ve records	% of the raw total	Activity Index	SE
Keninup	264	77.4	0.366	0.020
Balban	95	84.8	0.109	0.027
Warrup	173	92.0	0.233	0.024
Boyicup	28	87.5	0.042	0.011
Winnejup	45	86.5	0.077	0.023
Total	**605**	**83.5**		

Figure 4.4.14. Woylie activity index for each session at all Upper Warren PCS sites.

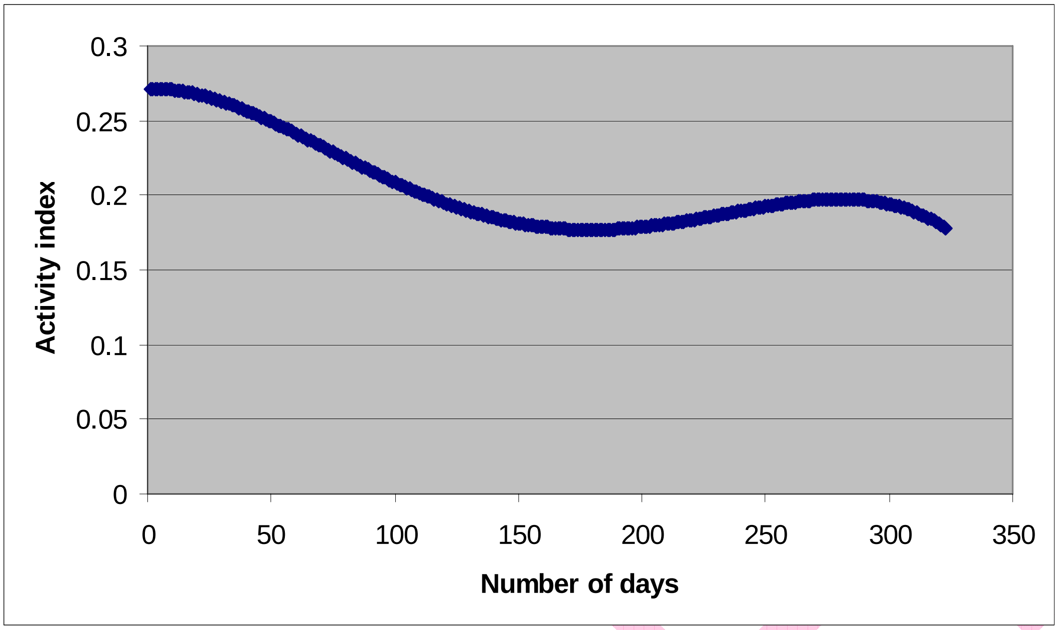


Figure 4.4.15. Temporal differences in the woylie activity index for Upper Warren PCS sites.

Koomal activity indices

Koomal were well represented at all sites during all sessions (Figure 4.4.16.). The percentage of total records used for the AI calculation was relatively high for all sites (Table 4.4.10.). This is reflective of possum prints not being readable on sandpads subjected to heavy rain. Presence of koomal foot prints were found in this study to be a reliable indicator of sandpad condition.

Differences in koomal activity levels were highly significant between sites ($p=0.0001$, $df=4$) Overall koomal mean activity was significantly higher at Balban than all sites, other than Winnejup. Winnejup, Warrup and Boyicup were similar in koomal number and Keninup had the lowest record of koomal (Table 4.4.10.). There was a significant temporal difference ($p<0.05$, $df=4$), and an apparent trend for activity to be higher through the summer months at all sites (Figure 4.4.17.)

There was a significant difference in the koomal activity pre and post baiting ($p=0.0231$, $df=1$) (Table 4.4.11.), with koomal activity increasing after baiting.

Table 4.4.10. Summary of koomal activity.

Site	No. +ve records	% of the raw total	Activity Index	SE
Keninup	187	85.4	0.269	0.034
Balban	389	96.5	0.512	0.046
Warrup	274	90.7	0.395	0.051
Boyicup	252	91.0	0.354	0.050
Winnejup	261	88.8	0.427	0.041
Total	**1363**	**91.2**		

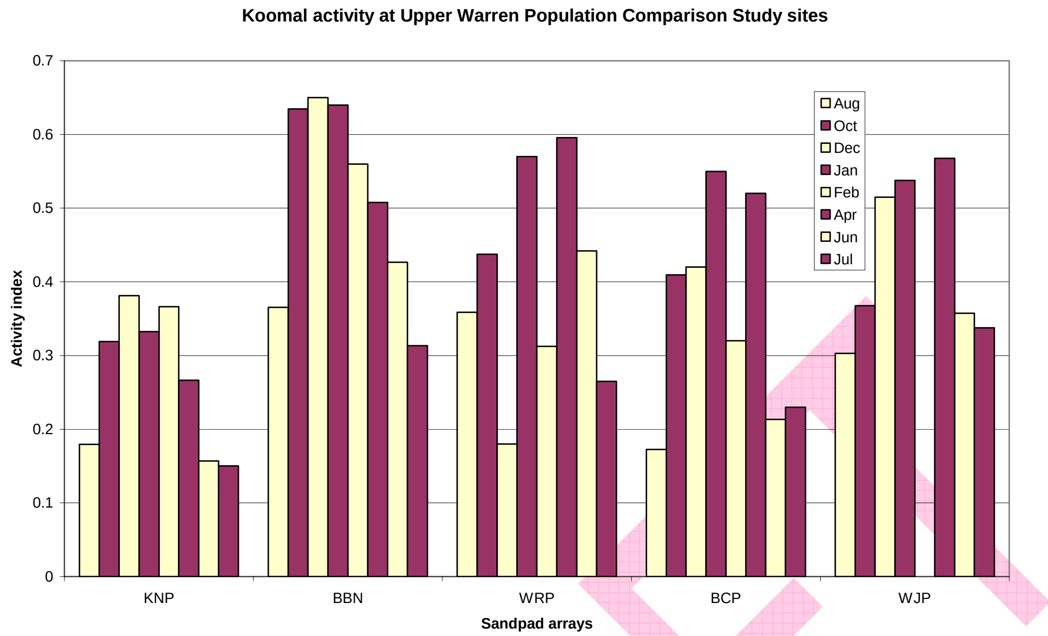


Figure 4.4.16. Koomal activity index for each session at all Upper Warren PCS sites.

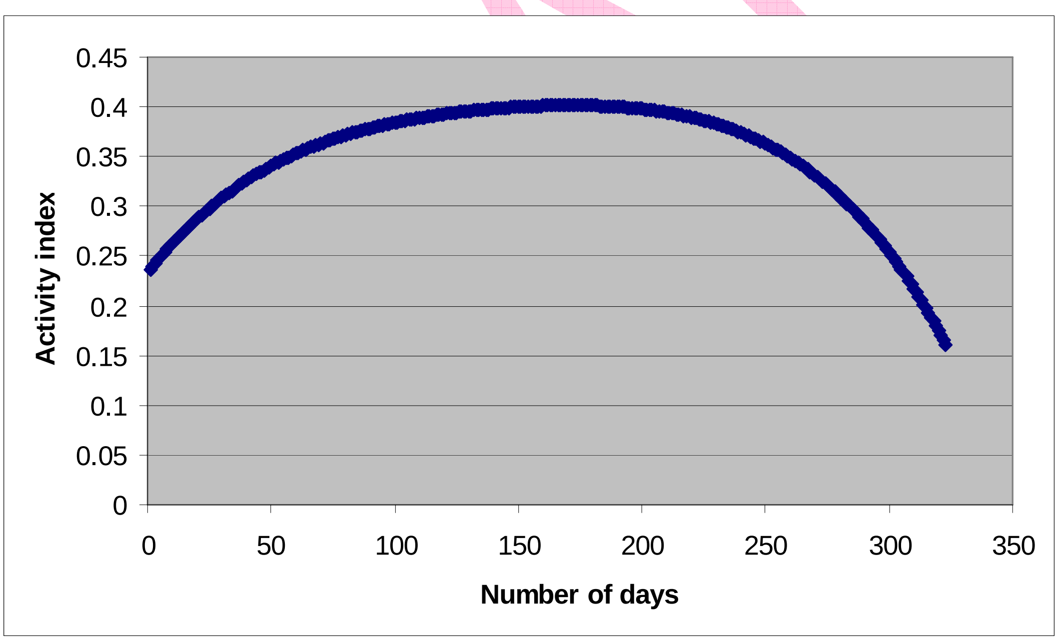


Figure 4.4.17. Temporal differences in the koomal activity index for Upper Warren PCS sites.

Macropod activity indices

Macropod activity does not distinguish between the western grey kangaroo, tammar and western brush wallaby records. Woylies were always recorded separately.

There was a significant difference between sites for macropod activity (p=0.0001, df=4), with Keninup highest (similar to woylie activity) and Winnejup and Boyicup the least (Table 4.4.12. and Figure 4.4.18.). There was a significant difference over time in macropod activity (p<0.05, df=4) and similar to koomal, the AI was higher over the summer period (Figure 4.4.19.).

There were significantly more macropod records post baiting (p=0.0019, df=1) (Table 4.4.12.), similar to koomal.

Table 4.4.11. Summary of macropod activity.

Site	No. +ve records	% of the raw total	Activity Index	SE
Keninup	252	75.2	0.345	0.042
Balban	231	87.2	0.301	0.026
Warrup	147	81.7	0.214	0.032
Boyicup	166	87.4	0.227	0.017
Winnejup	135	75.0	0.228	0.032
Total	**931**	**81.0**		

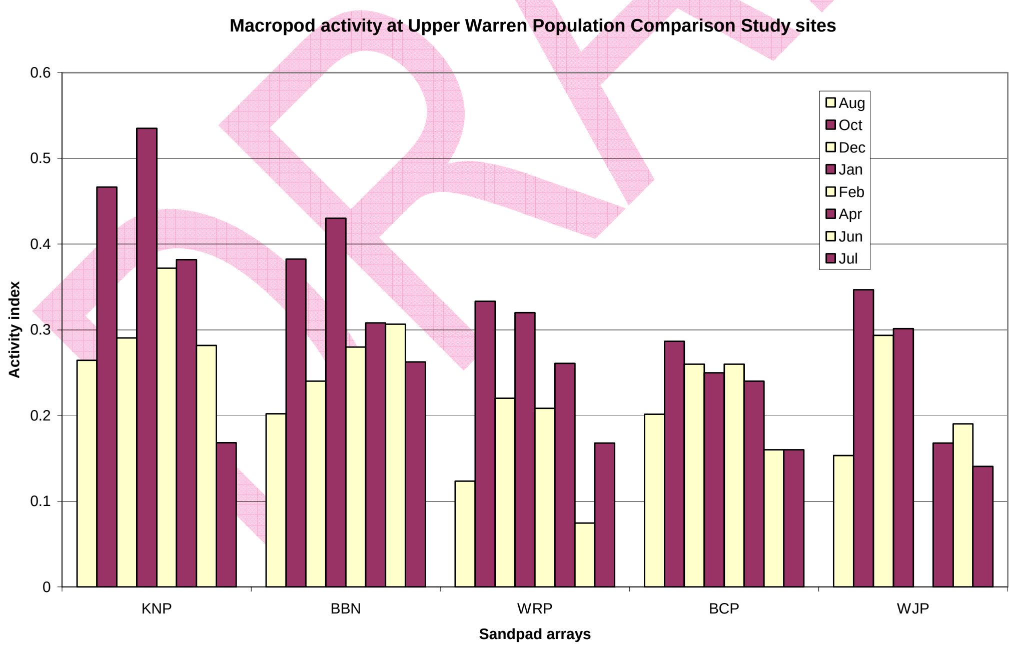


Figure 4.4.18. Macropod activity index for each session at all Upper Warren PCS sites.

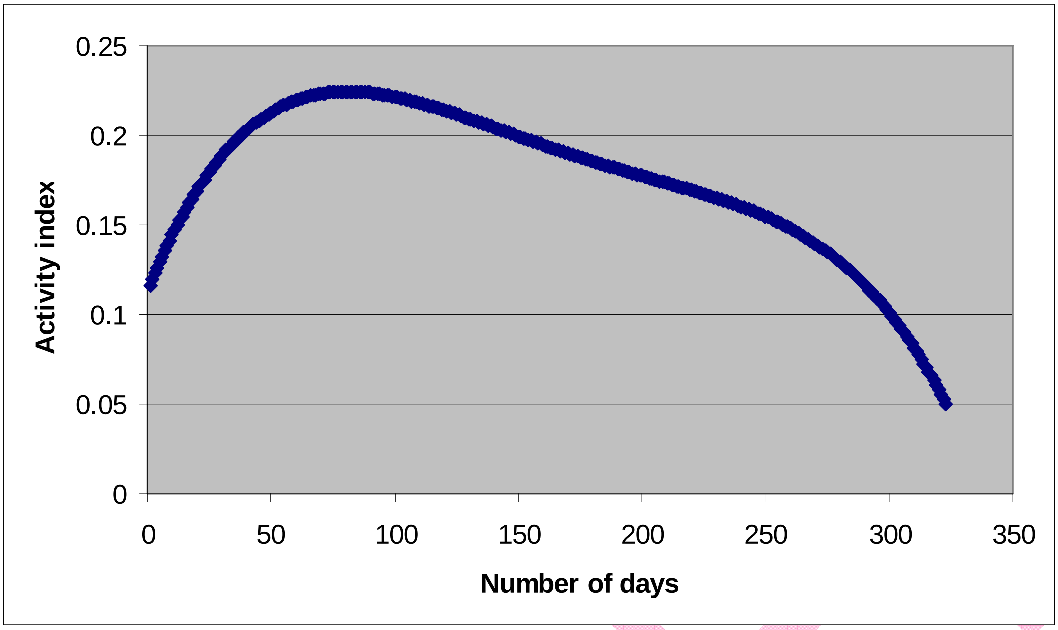


Figure 4.4.19. Temporal differences in the macropod activity index for Upper Warren PCS sites.

Table 4.4.12. Significance tests for the activity indices of introduced predators and native mammals derived from sandpad surveys at the Upper Warren PCS sites.

Cat

Source	DF	Mean Square	F Value	Pr > F
time covariate*	4	0.0047	3.16	0.0311
site	4	0.0016	1.07	0.3927
treat	1	0.0032	2.17	0.1534
site x treat	4	0.0016	1.06	0.3945
error	25	0.0015		

Fox

Source	DF	Mean Square	F Value	Pr > F
time covariate*	4	0.0102	3.70	<0.05
site	4	0.0072	2.62	0.0593
treat	1	0.0024	0.88	0.3581
site x treat	4	0.0038	1.37	0.2712
error	25	0.0028		

Chuditch

Source	DF	Mean Square	F Value	Pr > F
time covariate*	4	0.0266	8.70	<0.05
site	4	0.0357	11.67	<.0001
treat	1	0.0037	1.23	0.2786
site x treat	4	0.0030	0.99	0.4293
error	25	0.0031		

Woylie

Source	DF	Mean Square	F Value	Pr > F
time covariate*	4	0.0079	2.84	<0.05
site	4	0.1425	51.12	<.0001
treat	1	0.0035	1.27	0.2706
site x treat	4	0.0038	1.37	0.2710
error	25	0.0028		

Koomal

Source	DF	Mean Square	F Value	Pr > F
time covariate*	4	0.0696	9.80	<0.05
site	4	0.0651	9.17	0.0001
treat	1	0.0416	5.86	0.0231
site x treat	4	0.0099	1.40	0.2634
error	25	0.0071		

Macropod

Source	DF	Mean Square	F Value	Pr > F
time covariate*	4	0.0331	11.81	<0.05
site	4	0.0257	9.16	0.0001
treat	1	0.0336	11.99	0.0019
site x treat	4	0.0039	1.40	0.2638
error	25	0.0028		

*Average of Type I linear, squared, cubic and quadratic day value variables. Other source variables derived from Type III SS. Critical value is 2.76 for time covariate.

Predator activity in relation to woylie populations

Figures 4.4.20. - 4.4.25. relate the fox and cat activity indices derived from the sandpad surveys with the capture rates of woylies derived from the PCS trapping grids (Section 4.2 Demographics).

Keninup: The woylie capture rates (%) decreased from February 2007 in conjunction with an increase in the fox activity index (Figure 4.4.20.). Keninup had considerably higher woylie capture rates (%) compared to the other Upper Warren PCS sites

Balban: The decline in woylie capture rates has occurred generally in the presence of higher cat activity compared to the other sites, particularly at the beginning of the survey (Figure 4.4.21.). This cat activity however, was not significantly different at Balban compared to other sites. Balban was, the only site in a state of woylie decline during the study period. Similar to Keninup there was also a general increase in fox activity over time.

Warrup: Woylie capture rates fluctuated the least in comparison to other Upper Warren PCS sites (Figure 4.4.22.). Cat and fox activity was variable over time with no striking trends.

Winnejup and Boyicup: Very low (hence relatively variable) woylie capture rates over the period of the predator surveys make it difficult to relate this data to cat and fox activity (Figures 4.4.23. and 4.4.24.).

In general, the woylie activity indices calculated from the sandpads approximately related to the same trends observed in the capture rates derived from the PCS trapping grids in the same area. For example, relative activity levels between sites ranked similarly to the relative capture rates (i.e. greatest at Keninup, followed by Warrup, Balban, Winnejup and least at Boyicup) and the declining woylie trends at Balban are very similar between the two independent datasets (Figure 4.4.25.).

Koomal capture rates continued to increase over time at the Balban PCS site, with a dip in February 2007 in conjunction with a spike in the fox activity index (Figure 4.4.26.). A similar trend in koomal capture rates also occurred at other PCS sites. Koomal capture rates also increased in the Balban Upper Warren Fauna Monitoring transect from 7% to 36. 5% from Mar 2006 to Mar 2007.

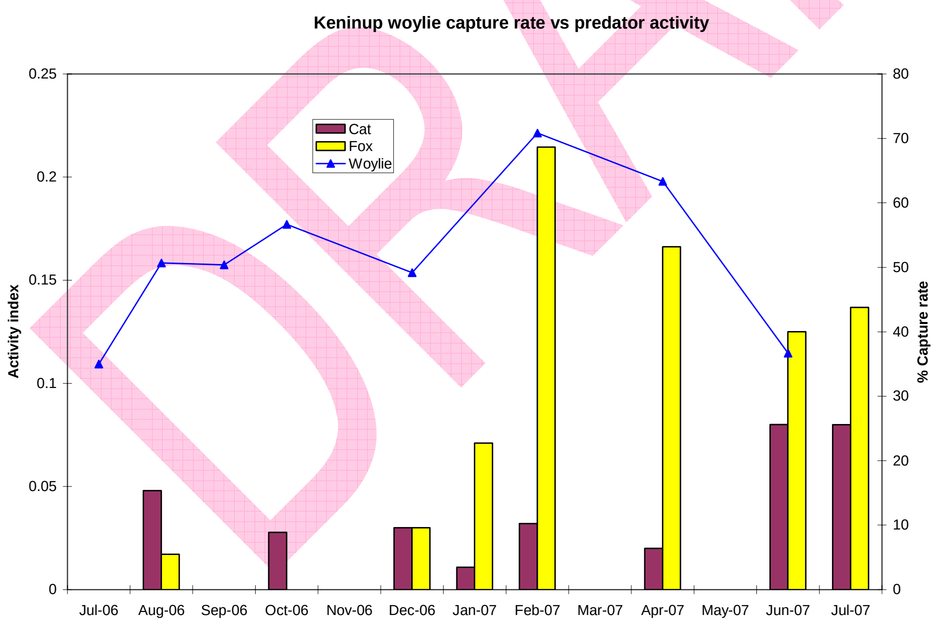


Figure 4.4.20. Keninup woylie capture rate in relation to the fox and cat activity index.

Balban woylie capture rate vs predator activity

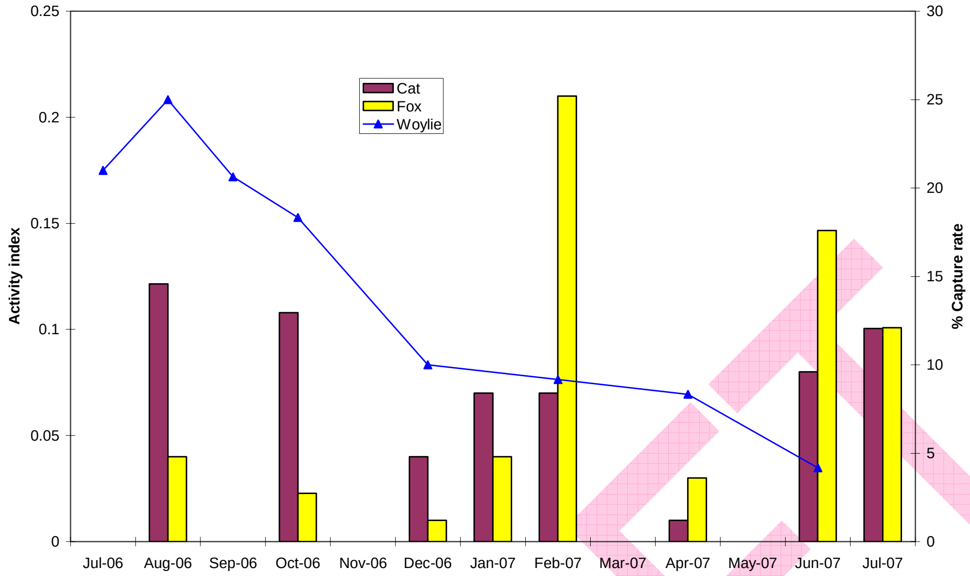


Figure 4.4.21. Balban woylie capture rate in relation to the fox and cat activity index.

Warrup woylie capture rate vs predator activity

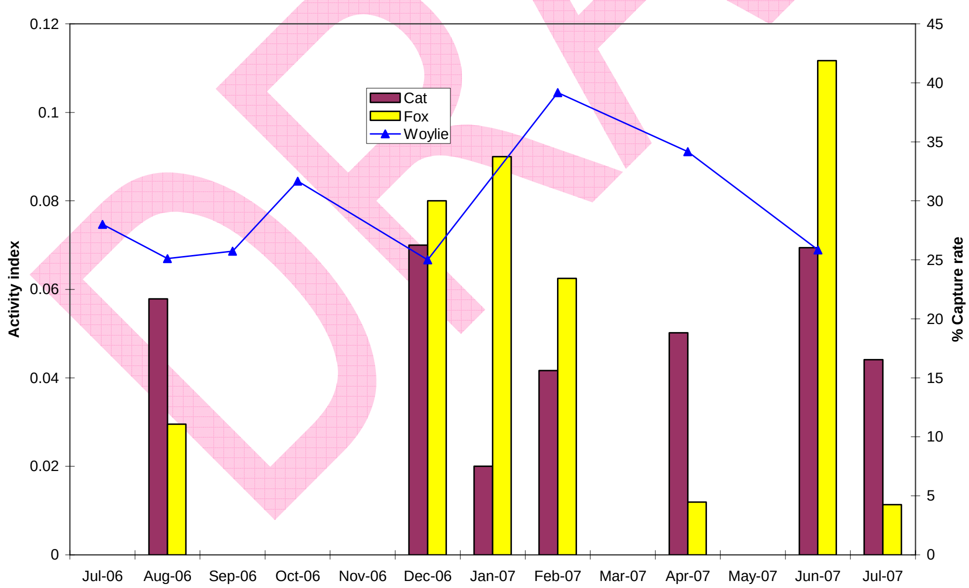


Figure 4.4.22. Warrup woylie capture rate in relation to the fox and cat activity index.

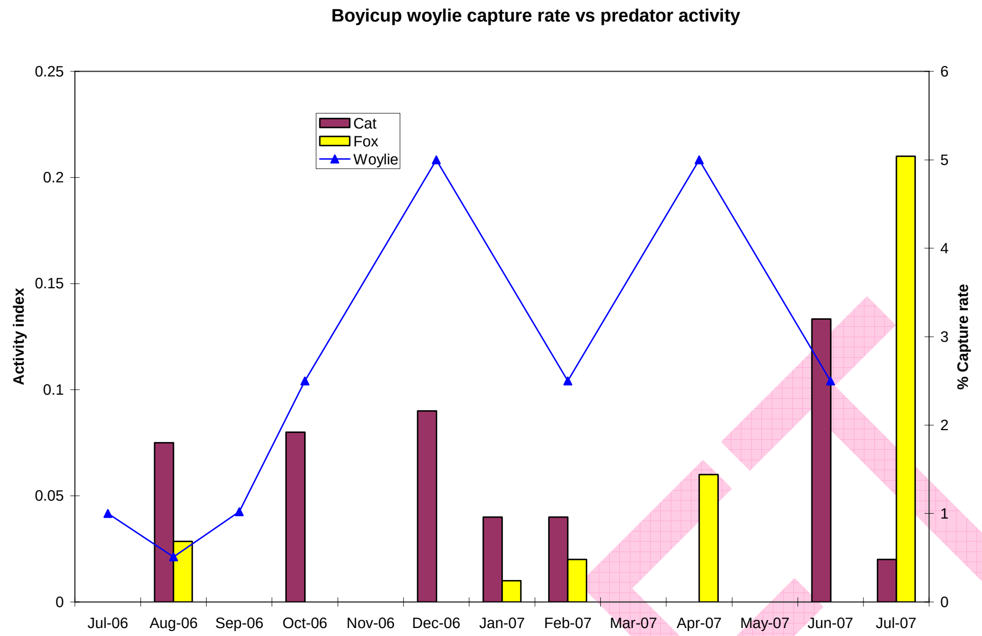


Figure 4.4.23. Boyicup woylie capture rate in relation to the fox and cat activity index.

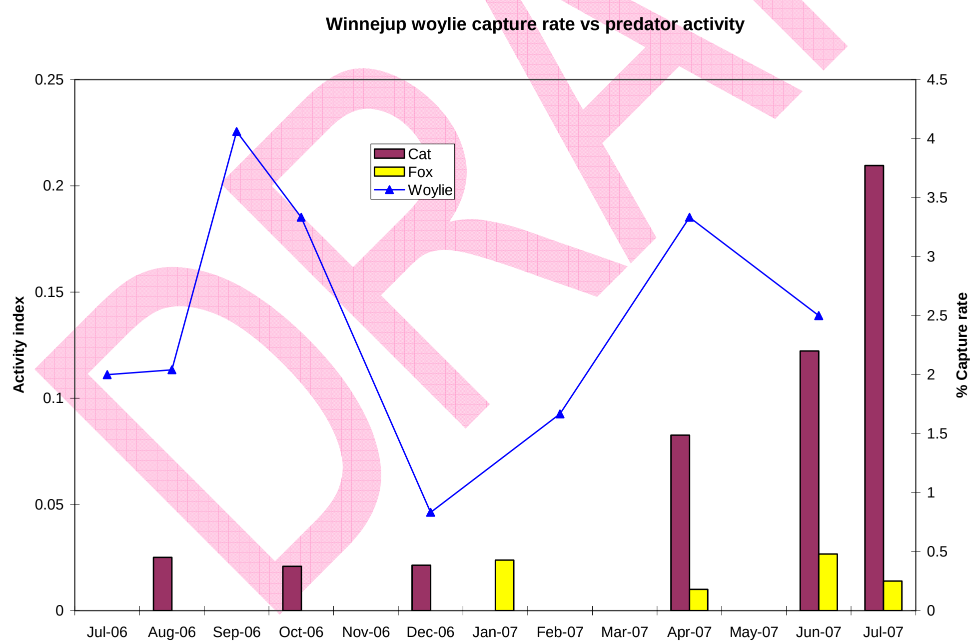


Figure 4.4.24. Winnejup woylie capture rate in relation to the fox and cat activity index.

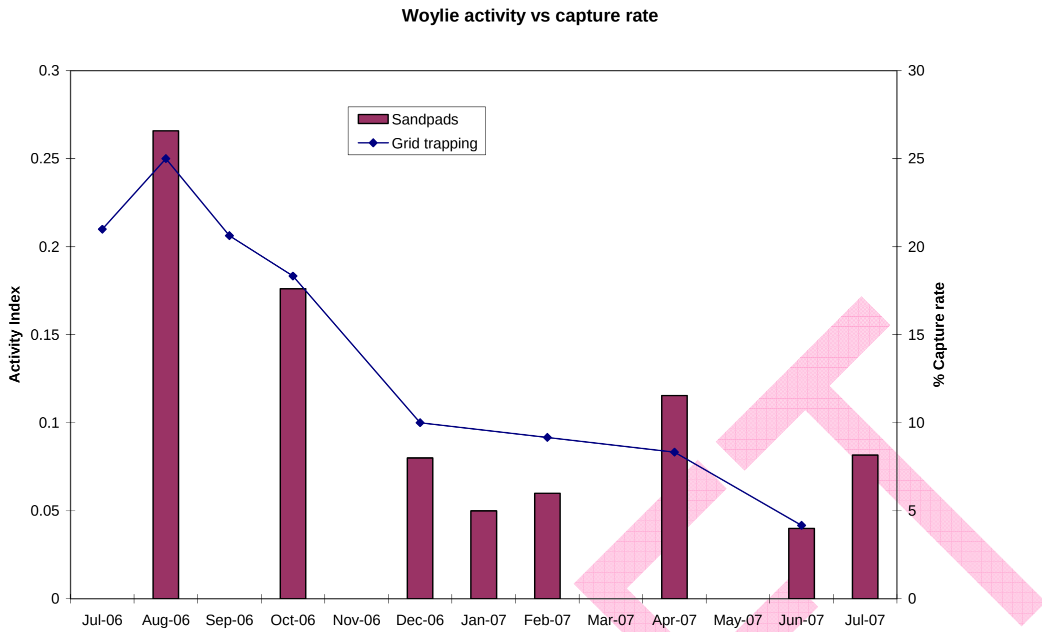


Figure 4.4.25. Woylie activity index in relation to capture rate at the Balban PCS site.

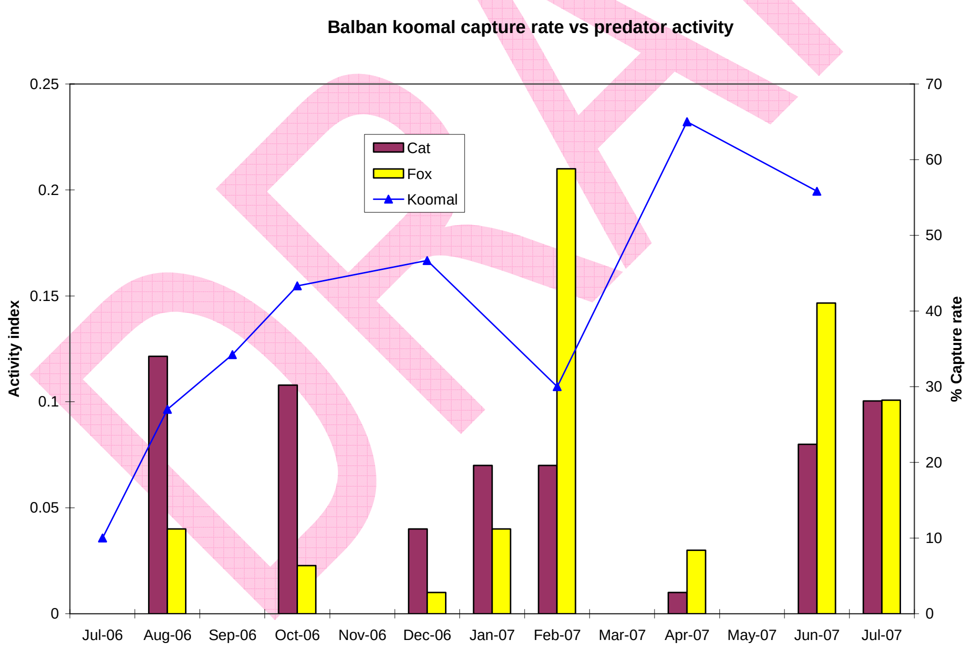


Figure 4.4.26. Balban koomal capture rate in relation to the fox and cat activity index.

4.4.3.4. Other results relating to methodology

A number of results from the sandpad surveys may be relevant to the survey methodology, and help to inform how future sandpad surveys may be conducted. Some of these are very briefly addressed here in point form.

Assessment of passive and active pad methods

- Using all available data and having adjusted for the difference in numbers of active and passive sandpads, the incidence of fox prints on passive sandpads was twice as high at that on active sandpads (6.8%, 3.4% respectively). Similarly cat activity was found to be greater on passive sandpads than active sandpads, however the difference was less marked, only 1.3 times higher (6.3%, 4.7% respectively). Conversely chuditch activity on active sandpads (15%) was 2.5 times that of passive sandpads (6.2%).
- There were inconsistencies between sites and observers on the level of detail recorded about predator behaviour on the sandpad. This detail was recorded more consistently in later surveys upon review of the results.
- Despite the large percentage of "not recorded" in most tables there are some clear trends in relation to the oil lure and FAP (Table 4.4.13.).
- Cats generally did not venture near the oil and there was a greater percentage that did not visit the FAP. Many cats were detected at the end of the sandpad opposite the FAP. Again this observation was not apparent until later in the study and hence this behavioural observation was often not recorded. In general cats walked directly across the sandpad.
- Of the 35% of foxes recorded on active pads there was less dissimilarity in oil visitation than for the other predator species. There was a slight bias to not visiting the FAP.
- There was a clear trend for chuditch to visit either the FAP or the oil, and often both. The chuditch would often also scrape or dig at the oil and defecate on the sandpad. Behavioural activity differed considerably between active and passive sandpads, with many footprints observed on active sandpads, often superimposed, compared to only one set of tracks on passive sandpads. The large size of the sandpads meant that clear identification of prints was still possible.

Table 4.4.13. Predator positive (+ve) or (-ve) visitation to oil lure and FAP.

Cat

Site	Oil (+ve) [dig at oil]		Oil (-ve)	Not recorded	Total	FAP (+ve)	FAP (-ve) [opp FAP]		Not recorded	Total
KNP	3	[1]	10	8	21	8	6	[3]	7	21
BBN	1	[0]	26	8	35	6	22	[7]	7	35
WRP	1	[0]	4	11	16	1	5	[4]	11	17
BCP	5	[3]	19	1	25	5	20	[5]	0	25
WJP	1	[0]	3	16	20	1	3	[3]	15	19
Total	11	[4]	62	44	117	21	56	[22]	40	117
%	**9.4**		**53.0**	37.6		**17.9**	**47.9**		34.2	

Fox

Site	Oil (+ve) [dig at oil]		Oil (-ve)	Not recorded	Total	FAP (+ve)	FAP (-ve) [opp FAP]		Not recorded	Total
KNP	12	[2]	18	4	34	13	17	[7]	4	34
BBN	10	[1]	8	2	20	5	13	[6]	2	20
WRP	2	[0]	2	10	14	2	2	[0]	10	14
BCP	4	[1]	3	2	9	2	5	[3]	2	9
WJP	0	[0]	3	3	6	0	3	[0]	3	6
Total	28	[4]	34	21	83	22	40	16	21	83
%	**33.7**		**41.0**	25.3		**26.5**	**48.2**		25.3	

Chuditch

Site	Oil (+ve) [dig at oil]		Oil (-ve)	Not recorded	Total	FAP (+ve)	FAP (-ve) [opp FAP]		Not recorded	Total
KNP	100	[47]	10	37	147	62	10	[0]	76	148
BBN	50	[22]	5	35	90	35	8	[0]	47	90
WRP	17	[2]	1	45	63	6	2	[0]	54	62
BCP	13	[1]	0	7	20	11	5	[0]	4	20
WJP	15	[0]	0	42	57	3	7	[0]	47	57
Total	195	[72]	16	166	377	117	32	0	228	377
%	**51.7**		**4.2**	44.0		**31.0**	**8.5**		60.5	

Scat collection

- A total of 147 scats were collected from the sandpads. Of these, 87 were fresh and collected during the survey and 59 were old, and collected during sandpad preparation on the first day of the survey.
- The majority of scats collected were from chuditch (e.g. 78/87 fresh scats). Only one definite (three possible) cat and 2 possible fox scats were collected.
- 76 of the 78 chuditch scats were collected from active pads. The scats were usually found on or adjacent to the oil lure.
- The number of scats on the sandpads (particularly chuditch) increased with the number of survey days, and hence is not independent. The greatest numbers of scats were

collected from sandpads during pad preparation. This suggests that the oil persists for sometime on the sandpads for the chuditch to continue digging at the oil and defecating.

General notes

- Cat and fox prints were often observed only within the wheel ruts of the sandpad.
- Fox urine scent was detected on some sandpads.
- Habitual patterns of fauna were recognized and may provide an insight to some extent of animal behaviour. The same records of fauna could be found on particular sandpads consistently. For example at Keninup, quenda activity was found on Pad 1 or 2 or both, on 62.5% of nights. Also at Keninup chuditch activity was observed on Pad 25 on greater than 50% of nights.
- In many instances sets of woylie prints were observed crossing a sandpad in a similar position each day. For example, Pad 4 at Keninup had a woylie crossing the pad lengthways on 81% of nights.

4.4.4. Discussion

Fox control

It is reasonable to expect that existing fox-baiting activities are effective to some extent in reducing, and possibly maintaining, fox numbers at levels lower than they would otherwise be. This has been shown elsewhere (Kinnear *et al.,* 1988; de Tores, 1999; Thomson *et al.,* 2000). Furthermore, evidence from the Upper Warren is also consistent with this; including reduced fox activity in baited forest relative to unbaited forest (J Rooney, 2001 Kingston Project Review (unpublished data)), and positive responses by native fauna in association with the commencement and/or increase in fox control (e.g. Burrows and Christensen, 2002; Morris *et al.,* 2001; Orell, 2004).

Nonetheless, it is evident from the records of fox-control since 1996 that the delivery has been highly variable in the Upper Warren. For example, the timing of the ground baiting in relation to aerial baiting has lagged by up to two months. The intervals between consecutive baiting sessions have ranged from 1 - 6 months for aerial baiting and 1 - 9 months for ground baiting. It follows that these variations and extended intervals are likely to increase the potential for the reinvasion of foxes into the managed forests and increase accordingly the predation pressure on the vulnerable native fauna that the fox control program is intended to protect.

Potentially more importantly, the timing of fox-baiting relative to when it is considered particularly effective, has also been variable. Despite a relatively more consistent *Western Shield* baiting program since 2002, extended baiting intervals have frequently occurred over the summer periods. For example, in 59% of cases (including five consecutive years from 2000 to 2004) there was no ground baiting conducted between January and March – when young foxes begin to disperse and when control is thought to be particularly effective in reducing fox numbers (Saunders *et al.,* 1995; Thomson *et al.,* 2000).

The effectiveness of fox baits may also be further compromised by heavy rainfall and wet conditions during and immediately after bait delivery. As a consequence of both the variability in the timing of aerial baiting and rainfall events only 30% of the years between 1997 and 2006 can be considered to have had a full complement of four presumed-effective baiting events per year.

In the absence of previous ongoing predator monitoring it is not possible to know directly what affects the variability in fox baiting may have had on predator numbers or how predators may relate to the woylie declines or any other native animals of interest. Extended periods of little or ineffectual baiting, are however, likely to result in periodic increases in foxes, which could consequently have substantial and longer-lasting impacts on some native species.

Reinvasion of foxes into the Upper Warren forests is likely to occur all year round, particularly given the proximity and distribution of agricultural sources for reinvasion. Due to the high reinvasion potential increased baiting frequency (i.e. reduced baiting intervals) and strategic considerations of the timing of baiting may be particularly beneficial to native fauna conservation. For example, the timing of baiting relative to fox biology (i.e. dispersal periods; Saunders *et al.,* 1995; Thomson *et al.*,

2000) and avoiding wet conditions are likely to have significant impacts. Improvements may also be achieved through spatial considerations such as varying baiting frequency and/or density relative to proximity to unbaited and agricultural land. de Tores (1999) recommends a baiting frequency of six times per year at the interface between baited and unbaited areas.

A more complete understanding of the factors influencing the effectiveness of fox control, if used operationally, could potentially achieve substantial improvements without significant impact on existing resources. The monitoring of predator (and prey) populations is essential in substantiating the effectiveness of these control measures and enables an active adaptive management approach that enables further improvements over time.

General temporal trends in AI

There were significant changes in the activity indices over time for all species examined. Some of these changes were consistent with expectations of seasonal differences in activity relating to reproduction, offspring dispersal and food resources. Multiple years of comparable data would be able to differentiate cyclical from other longitudinal changes and trends in activity indices, however, this is beyond the capacity of the one year of data collected in this study.

Cat

Cats persisted at all PCS sites and activity indices (AI) were not significantly different between sites. Cat AI did not differ significantly pre and post fox-baiting. This is expected since fox baits are not attractive to cats (Algar and Burrows, 2004) and have been found ineffective in control of feral cats (Christensen and Burrows, 1994; Risbey *et al.,* 1997). The overall increase in cat AI in the latter half of the study was influenced by a particularly large increase at Winnejup. The extent to which significant temporal changes in cat AI may be due to seasonal or longer-term trends remains unknown.

Fox

While the overall Upper Warren average AI for the fox and cat were similar (0.054 and 0.058 respectively), the variability in the fox AI's within sites generally tended to be greater. Higher population turnover and immigration from non-baited areas replenishing reduced fox numbers in baited areas is likely to account for at least some of this variance.

Differences in the fox AI between sites was marginally significant. Keninup and Balban had particularly high fox AI's while Boyicup and Winnejup were relatively low. These AI's corresponded with the contemporary relative abundances of woylies at these sites. Fox AI's may also be related to some extent to the general levels of geographic exposure and proximity to unbaited forest areas and agricultural land, where fox numbers are likely to be higher (i.e. recruitment sources). Keninup has the greatest potential for immigration of foxes due to both proximity and large interface between forest and agricultural land. Balban also has a large unbaited corridor allowing for potentially greater reinvasion.

The temporal peak of fox AI at Keninup and Balban in February is consistent with the anticipated seasonal increase in fox activity associated with the independence and dispersal of fox subadults (McIntosh, 1963; Ryan, 1976). Over this period there were only three records of relatively small fox prints (two in January and one in April 2007). The lack of an apparent corresponding peak at the other Upper Warren PCS sites may be due to the varying potential for reinvasion between sites or could be related to the reduced effective sampling on these sites as a result of the heavy rainfall during the surveys and the inability (resource limitations) to resample these sites the following week, as was done at Keninup and Balban.

The significant increase in fox activity over the 12-month study (especially after February 2007) was particularly pronounced at Keninup (where woylie numbers were greatest), Balban (concurrently declining woylies) and later (July 2007) at Boyicup (woylies previously declined and sustained low). The possibility that the fox control since February 2007 has not been particularly effective is supported by the wet conditions associated with the June 2007 baiting event (Table 4.4.1.). However, the results must be viewed cautiously as activity levels may also be affected by seasonal behavioural changes, such as breeding behaviour (Phillips and Catling, 1991; Thomson, 1992).

Previous sandpad survey results within the Upper Warren region (1996-1999; 2005) found fox activity to be significantly higher in the unbaited versus baited areas and the fox activity on the forest / agricultural boundary to be significantly higher than the core (both baited and unbaited) (J Rooney, 2001 Kingston Project Review (unpublished data)). Unlike the current study, surveys were conducted only in September, when fox populations are considered the most stable (de Tores, 1999). Whether the observed increases in fox activity in this study are seasonal, periodic and/or part of a longer-term trend or cycle can only be verified with longer-term data.

Assuming that fox control remained effective to some extent during this study (discussed above), the insignificant difference between fox activity pre and post baiting found in this study is best explained by insufficient statistical power due to limited sampling, low fox print encounter rates and high variance. It is also possible that i) density-dependent effects on fox activity may mask reductions in actual fox numbers immediately after fox control. Fox control elsewhere has resulted in static or elevated AI due to changes in the activity of remaining foxes (Allen *et al.,* 1996). For example, foxes may extend the length of road walked per night due to altered territorial boundaries, and/or ii) as discussed previously fox reinvasion/recovery may be rapid, despite fox-control activities being effective in reducing general fox densities as has been shown in previous studies (Kinnear *et al.,* 1988).

It is also possible that baiting effectiveness may be reduced by non-target species, (e.g. chuditch, koomal and varanids), consuming baits and hence reducing overall bait availability to foxes (Algar *et al*., 2007). On this basis it is possible that baiting effectiveness in the Upper Warren is also influenced by the relatively high numbers of koomal in particular, as well as other native nontarget species (Chapter 3 Meta-analysis and Section 4.2 Demographics).

Chuditch

The significant site differences in chuditch AI generally corresponded to relative woylie abundance (i.e. highest at Keninup and lowest at Boyicup). Chuditch were found to be associated with a number of woylie mortality events based on forensic odontology, but chuditch are more likely to have been involved as secondary scavengers than a primary predator. There is no clear evidence to implicate chuditch in the woylie decline (Section 4.3 Survival and Mortality).

The significant temporal differences in chuditch AI were likely to be related to seasonal differences in chuditch behaviour. The pattern of chuditch activity is highly characteristic of a cyclical phenomenon and is consistent with our understanding of the biology and behaviour of the chuditch (Soderquist and Serena, 2000; Morris *et al.,* 2003). For example, the AI peak around May coincides with mating when males are highly mobile in the search for mates (Serena and Soderquist, 1989; Morris *et al.,* 2003).

Woylie

The strong association found in this study between woylie AI's (derived from sandpad methods) with capture rates (derived from concurrent WCRP trapping) is consistent with previous findings for the Upper Warren (Wayne, 2006). This relationship provides supporting independent evidence about the extent and magnitude of recent woylie declines. Furthermore, it provides an alternative or complementary non-invasive and relatively low cost means of monitoring populations.

Predator / Prey relationships

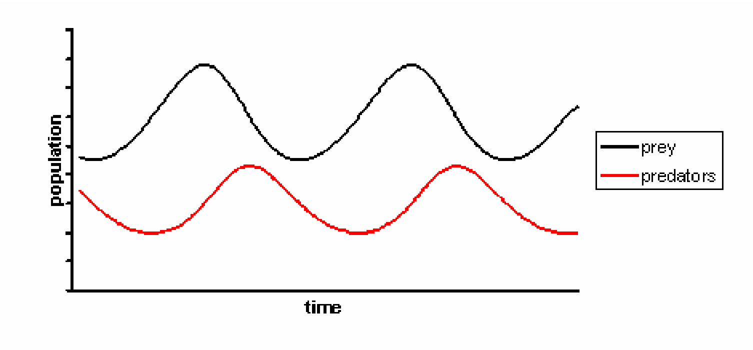
The fact that woylie populations have not shown a decline at effectively predator-free Karakamia and South Australian islands (Venus Bay Island A and St Peter's Island) supports the hypothesis that predators may be a factor in the woylie decline.

The extent to which predators such as the cat and fox are involved in recent woylie declines within the Upper Warren cannot be definitively answered with only 12-months of comparable predator activity data. It does, however, provide some preliminary clues, particularly when considered with other supporting evidence. For example, there may be a temporal association between increased cat activity and the decline of woylies at Balban. This is further implicated by i) the high incidence of cats associated with mortalities of radio-collared woylies at Balban during the period of woylie decline (seven out of eight cases) (Section 4.3 Survival and Mortality); ii) cat activity was greatest at Balban where woylies were declining and least where woylie abundance was relatively high and stable (i.e.

Keninup and Warrup); and, iii) Yendicup and Yackelup sandpad survey results, which commenced in 2000 and show a trend of increasing cat activity in association with declining woylie activity (Bruce Ward and Graeme Liddelow, pers. comm.).

However, evidence which is seemingly inconsistent with predators being a primary cause of decline is despite the similarities between Keninup and Balban in relation to cat and fox AI, Keninup woylies did not decline where they did so at Balban. Furthermore, the survivorship results from Keninup do not directly support fox as responsible because only two mortalities occurred during the period of increased fox activity from February – July 2007, and neither was associated with fox. (one cat, one raptor) (Section 4.3 Survival and Mortality).

Determining the nature of the relationship between predator activity and woylie abundance (and declines) is made more difficult in the fact that relationships between predator and prey abundance can be nonlinear and complicated by temporal differences (lags) (Figure 4.4.27- Theoretical model of predator / prey abundance over time).



(Source: http://commons.wikimedia.org/wiki/Image:Lotka-Volterra.png)

Figure 4.4.27. Lotka-volterra predator-prey model

For example, at any one point in time the predator densities at a site with low prey densities may be high because of previous or current exploitation of the prey resource, or low because the site can no longer sustain the predator densities that it previously did. As a result, suitable data over an extended period is required to reliably determine the nature of any associations between predator and prey abundances. To do this the study period needs to encompass sufficient time before, during and after a prey or predator population transition between low and high density or vice versa.

Hence, there is currently insufficient data and evidence within the Upper Warren to confidently determine what extent predators are involved in the woylie decline, however continued predator monitoring remains integral to rectifying this.

Koomal

If predators were the primary cause for woylie declines then it would be likely that other, similarly-sized prey species may also decline. Koomal AI was substantially higher than any other species detected on the sandpads. Therefore there remains substantial opportunity for introduced predators to encounter koomal on the ground. Despite this, koomal capture rates remained stable or increased during the same period that woylies have declined (Chapter 3 Meta-analysis and Section 4.2 Demographics). This was particularly obvious at Balban where there was a decline in woylie and an increase in koomal capture rates. Bearing in mind species differences in their vulnerability to predation, the koomal capture and sandpad data do not provide supporting associative evidence that predation may be a primary cause of the woylie declines.

Considerations of the use of a predator activity index (AI) in this study

The use of an activity index, such as has been used in this study, is considered a more conservative and more appropriate means of measuring predators from sandpad data, than deriving an estimate of predator abundance. This is foremost because fewer assumptions and interpretations of the sandpads and data are required to derive an activity index. Furthermore, a predator activity index can be used as a simple estimation of the probability of risk that a woylie may encounter a predator, which in itself is a useful means of considering associations between predators and woylie declines.

It is important to emphasize the limitations of the AI data derived from only eight sandpad survey sessions within a 12-month period. While these have provided substantial and valuable insight, a more extensive dataset collected over multiple years would be required to improve the statistical power of analyses, thus more confidently test the validity of these trends and particularly any possible associations with other factors (e.g. woylie abundance). The low and relatively variable detection rates of foxes and cats in particular, also limit the power of analysis. As such, the results to date need to be regarded accordingly – i.e. preliminary, and in many cases statistically untested patterns and associations.

This study found that in many cases both foxes and cats directly crossed over the sandpads in the wheel ruts. The fact that footprints were often completely obscured once a vehicle had passed over the sandpad, as a result of the prints being confined to the wheel ruts, reaffirms the merit in excluding sandpads from analyses that have been disturbed by vehicles in order to avoid sampling biases. Nonetheless, some sampling biases will still persist given the non-random nature of vehicle disturbance (i.e. more common close to farmland and generally more prevalent in some areas than others). In the case of this study, vehicle disturbance at Keninup was particularly high, especially close to private property. As a result, cat and fox AI at Keninup is likely to be particularly conservative.

Operational Considerations

- The monitoring of predator activity within the Upper Warren has ceased as part of this study. However, there are compelling reasons for continuing some level of predator monitoring within the Upper Warren region. In particular, the value of the information derived from predator monitoring is potentially critically important to the diagnosis of woylie declines, woylie recovery and ongoing fauna conservation and management more generally. Furthermore, given that the monitoring infrastructure is now established the costs to continue monitoring are relatively small. To improve the analytical power of the data, future predator monitoring should factor in the low detection rates and high variance by increasing the number of sandpads per site and particularly the number of sampling nights per survey session. The latter is especially useful for overcoming the problems and influence of undesirable weather such as heavy rain on an otherwise limited dataset.
- Weather, especially rain, had the greatest impact on data collection (i.e. complete or partial destruction of animal prints and signs), resulting in a significant reduction in sample size (i.e. 20% survey days lost due to heavy rain alone). Extended survey sessions would compensate for weather disruption.
- A less subjective method for determining sandpad decipherability is required. For example, an approach similar to that of Allen *et al.,* (1996) could be used, whereby a mark is left by the assessor on the sandpad to gauge pad condition. The same mark is placed consistently in the same place on each sandpad and whether this print is discernable the next day determines whether the pad is included in analysis. This would help account for sand condition, rain and wind effects.
- Consistency in pad preparation and recording between observers is particularly important. Efforts are required to ensure training, clear instruction and continual feedback is provided to maintain consistency.
- Development and use of other techniques to quantify predator activity, such as the use of sensor cameras and more particularly hair DNA analysis techniques. This would help determine baiting effectiveness and whether the increase in predator activity post-baiting is a result of behavioural changes or actual changes in fox density.'
- The results of this survey suggest that the passive sandpad method is sufficient for assessing activity of cats and foxes. However, a combination of FAP and "Pongo" (mixture of cat urine and faeces) may have provided different results than found with a FAP and oil lure. This combination of communicative and food lure, respectively, may

send mixed signals to cats and may explain why avoidance behaviour was observed. Ideally the FAP should be hidden and not clearly visible as in this study, and used in combination with another communicative lure, such as PONGO for best results (Dave Algar, pers. comm.). Due to the difficulties associated with the sourcing and use of Pongo it is still recommended that a passive method be used for future sandpad surveys in the Upper Warren. It is important that the sandpad covers the full width of the road from batter to batter to ensure prints are not missed. Not using lures associated with active sandpads reduces the cost and time required to prepare and conduct the predator surveys. It also reduces the potential for any avoidance behaviour (shyness), learned or territorial responses. As each day is independent, survey days within a session do not necessarily need to be consecutive which is logistically advantageous (i.e. can accommodate for weekends).

- Sandpad surveys can have multi-purpose value. They can be used as a non-invasive method for detecting presence of large mammals, such as macropods, difficult to trap species such as the numbat, as well as the detection of less common species. An example for the latter is quenda at Balban where only a very small number (four) have been captured over the history of trapping at the site, compared to 11 records from sandpads. Hence, recording of other species information and description of activity during predator surveys can be beneficial for other purposes. E.g. measurement of 1080 bait interference by non-target species and consideration and assessment of its potential influence on bait availability and control effectiveness.

4.4.5. Future work

The predator sandpad monitoring surveys have been suspended until the determination of the requirements for the next stage of the project. Expectation is that the Donnelly District will continue the surveys to some extent for general monitoring purposes once requirements for the woylie program are complete.

The refinement of the analyses of existing data, the development and relating to other available evidence, comparison of methodologies and results with mesopredator projects, and the subsequent publication of the results in a peer-reviewed journal, remains the current priority for this component of the WCRP.

4.4.6. Conclusions

- Fox control activities have been highly variable in the Upper Warren since 1996. More strategic spatial and temporal considerations of fox control, particularly in relation to fox biology, would be expected to deliver substantially better outcomes with negligible impact on existing resources.
- Without concurrent predator activity/abundance data collected over multiple years, it is not possible to know what the consequence of the baiting variability has been on predators nor is it possible to directly relate predators to woylie declines. Indirectly, however, the variability in fox control prior to the woylie declines in the Upper Warren is not strikingly different to baiting activities during or since the commencement of woylie declines.
- Fox activity did not differ significantly between pre and post fox-baiting. The most likely explanation for this is as a result of the insufficient statistical power due to limited sampling, low fox print encounter rates and high variance. Increasing the number of sandpads and/or number of sampling days within survey sessions is recommended to increase statistical power, as well as reduce the impact of adverse weather events.
- Ongoing monitoring of predator activity and/or abundance would be extremely valuable and highly recommended for the Upper Warren and other areas where predator control is conducted and fauna conservation is considered a high priority. Advantages include the ability to;

  i) monitor the effectiveness of predator control efforts and alert managers if/when an

issue arises.
ii) relate changes in native fauna directly to predator activity/abundance in the region.
iii) develop a better understanding of the factors associated with spatial and temporal differences in predator activity/abundance, such as discriminating seasonal and longer-term trends in predators.
iv) develop a better understanding of the predator-prey interactions at the population level.

- The significant temporal differences in activity observed for all species in this study cannot be satisfactorily explained without longer-term data (i.e. seasonal and longer-term trends, predator-prey interactions, etc).
- Although overall there were no significant site differences cat activity was substantially less at sites with stable and high woylie abundance and greatest at Balban where woylies were declining. The preliminary evidence remains consistent with cats potentially having a role in the decline of woylies, however, it is premature and there is insufficient data to determine the strength or nature of this association.
- There was a significant difference in fox activity between PCS sites, which generally was positively associated with woylie numbers. A similar trend was also observed for chuditch. There is no compelling evidence within the preliminary data that supports (or refutes) that foxes or chuditch are the principal cause for woylie declines.
- The general increasing trend in fox activity from February 2007, (when fox dispersal occurs) may be indicative of limited effectiveness in fox control during this period. Whether this is part of a longer-term trend remains to be seen.
- The high prevalence of koomal activity on sandpads indicates the potential risk of this species to predation. Despite this, koomal have not declined in association with woylies. This reduces the likelihood that foxes, in particular, may be principally involved in woylie declines given that koomal are also susceptible to fox predation (Morris *et. al.,* 1995). The extent to which cats predate koomal remains uncertain but it has been observed in the past (Wayne *et. al*., 2005).
- If predation is centrally involved in the woylie decline it is possible other underlying causes may predispose woylies to an increased risk of predation given that other prey species such as koomal have not also declined.
- It is recommended that an entirely passive sandpad methodology be used in the future. Cat activity on active pads was similar to passive pads, and of those pads that were active, most cats did not approach the oil and FAP.

4.4.7. Acknowledgements

This work was done in collaboration between Science Division and Donnelly District. We would like to thank John Rooney for his roles in the initial stages of the project, in particular the organisation and establishment of the sandpads and the substantial challenges associated with ensuring that the sand used and the sandpad establishment did not introduce *Phytophthora.* Donnelly District (DEC) employees established the sandpads under the direction and co-ordination of John Rooney, Ian Wilson, Julia Wayne and Brian Moss. We would also like to thank all personnel involved in the field monitoring and the numerous volunteers that assisted.

4.4.8. References

Algar, D. and N. D. Burrows. 2004. Feral cat control research: Western Shield review-February 2003. Conservation Science Western Australia **5(2)**: 131-163.

Algar, D., G. J. Angus, M. R. Williams, and A. E. Mellican. 2007. Influence of bait type, weather and prey abundance on bait uptake by feral cats (*Felis catus*) on Peron Peninsula, Western Australia. Conservation Science Western Australia 6(1): 109-149.

Allen, L., R. Engeman and H. Krupa. 1996. Evaluation of three relative abundance indices for assessing dingo populations. Wildlife Conservation **23**:197-206.

Burbidge, A. A., and N. L. McKenzie. 1989. Patterns in the modern decline of Western Australia's vertebrate fauna: causes and conservation implications. Biological Conservation **50**:143-198.

Burrows, N. D., and P. E. S. Christensen. 2002. Long-term trends in native mammal capture rates in a jarrah forest in south-western Australia. Australian Forestry **65**:211-219.

Cherriman, S. C. 2007. Territory size and diet throughout the year of the Wedge-tailed Eagle *Aquila audax* in the Perth region, Western Australia. Honours Thesis, Murdoch University.

Christensen, P., and N. L. Burrows. 1994. Project Desert Dreaming: the reintroduction of mammals to the Gibson Desert, Western Australia. In 'Reintroduction Biology of Australian and New Zealand Fauna'. Ed. M. Serena. Sydney, 199-207.

de Tores, P. 1999. Control and ecology of the Red Fox in Western Australia – prey response to 1080 baiting over large areas. 1997-1998 Report to Environment Australia National Feral Animal Control Program.

EPA / QLD PWS. 2007. Procedural Guide-Using the Allen Activity Index to measure wild dog activity. P 1-5.

Kinnear, J. E., M. L. Onus, and R. N. Bromilow. 1988. Fox control and rock-wallaby population dynamics. Wildlife Research **15**:435-450.

McIntosh, D.L. 1963. Reproduction and growth of the fox in the Canberra district. CSIRO Wildlife Research **8**:132-141

Morris, K., B. Johnson, P. Orell, G. Gaikhorst, A. Wayne, and D. Moro. 2003. Recovery of the threatened chuditch (*Dasyurus geoffroii*): A case study. Pages 435-451 in M. Archer, M. E. Jones, and C. R. Dickman, editors. Predators with Pouches: The Biology of Carnivorous Marsupials. CSIRO Publishing, Melbourne.

Morris, K., B. Johnson, J. Rooney, and C. Ward. 2001. The short-term impacts of timber harvesting and associated activities on the abundance of medium-sized mammals in the jarrah forest of Western Australia. Pages 60-70 in J. L. Craig, N. Mitchell, and D. A. Saunders, editors. Nature Conservation 5: Conservation in the Production Environments: Managing the Matrix. Surrey Beatty and Sons, Sydney.

Morris, K., P. Orell, and R. Brazell. 1995. The effect of fox control on native mammals in the jarrah forest, Western Australia. Pages 177-181. 10th Australian Vertebrate Pest Control Conference. Department of Primary Industry and Fisheries, Hobart.

Orell, P. 2004. Fauna monitoring and staff training: Western Shield review – February 2003. Conservation Science **5**:51-95.

Peery, M. Z., S. R. Beissinger, S. H. Newman, E. B. Burkett, and T. D. Williams. 2004. Applying the declining population paradigm: Diagnosing causes of poor reproduction in the marbled murrelet. Conservation Biology **18**:1088-1098.

Phillips, M. and P. C. Catling. 1991. Home range and activity patterns of Red Foxes in Nadgee Nature Reserve. Wildlife Research **18**:677-686.

Risbey, D. A., M. C. Calver, and J. Short.1997. Control of feral cats for nature conservation. I. Field tests of four baiting methods. Wildlife Research **24**:319-326.

Rooney, J. 2001. Kingston Project Review Report (unpublished data). Preliminary results from sand pad surveys: a comparison of fox and other mammalian activity between a baited and unbaited area of Jarrah forest associated wit the 'Kingston Project'.

Ryan, G. E. 1976. Observations on the reproduction and age structure of the fox, Vulpes vulpes L., in New South Wales. Australian Wildlife Research **3**:11-20.

SAS Institute I. 2006. Base SAS 9.1.3 Procedures Guide, 2nd edition. SAS Publishing, Cary, NC.

Saunders, G., B. J. Coman, J. E. Kinnear, and M. L. Braysher. 1995. Managing Vertebrate Pests: Foxes. Australian Government Publishing Service, Canberra.

Schmitz, A., and M. Copley. 1997. Karakamia Sanctuary. Landscope 13(2):17-22.

Serena, M., and T. R. Soderquist. 1989. Spatial organisation of a riparian population of the carnivorous marsupial *Dasyurus geoffroii*. Zoological Society of London **219**:373-383.

Soderquist, T. R., and M. Serena. 2000. Juvenile behaviour and dispersal of Chudich (*Dasyurus geoffroii*) (Marsupialia : Dasyuridae). Australian Journal of Zoology **48**:551-560.

Start, A. N., A. A. Burbidge, and D. Armstrong. 1995. Woylie recovery plan. Wildlife Management Program No.16. Western Australian Department of Conservation and Land Management and South Australian Department of Environment and Natural Resources, Perth.

Start, A. N., A. A. Burbidge, and D. Armstrong. 1998. A review of the conservation status of the woylie (*Bettongia penicillata ogilbyi* (Marsupialia: Potoroidae) using IUCN criteria. CALMScience **2(4)**:277-289.

Thomson, P. C. 1992. The behavioural ecology of dingoes in north-western Australia. II. Activity patterns, breeding season and pup rearing. Wildlife Research **19**: 519-530.

Thomson, P. C., N. J. Marlow, K. Rose, and N. E. Kok. 2000. The effectiveness of a large-scale baiting campaign and an evaluation of a buffer zone strategy for fox control. Wildlife Research **27**:465-472.

Wayne, A. F., A. Cowling, C. G. Ward, J. F. Rooney, C. V. Vellios, D. B. Lindenmayer, and C. F. Donnelly. 2005. A comparison of survey methods for arboreal possums in jarrah forest, Western Australia. Wildlife Research **32**:701-714.

Wayne, A. F., I. Wilson, J. Northin, B. Barton, J. Gillard, K. Morris, P. Orell, and J. Richardson. 2006. Situation report and project proposal: identifying the cause(s) for the recent declines of woylies in south-western Australia. A report to the Department of Conservation and Land Management Corporate Executive. Pages 1-28. CALM, Perth, Western Australia.

4.5. Resources

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Abstract

The availability, abundance and suitability of resources such as food have been identified as a potential factor in woylie declines. While the woylie is known to be mycophagous (i.e. hypogeal and epigeal fungi are a major component of the diet), no comprehensive studies have been undertaken examining temporal and spatial variation in diet and food resource availability. The food resources component of the Woylie Conservation Research Project (WCRP) is being addressed through a collaborative PhD research project between Murdoch University and the Department of Environment and Conservation. Preliminary surveys investigating fungi availability and the methods for assessing sporocarp abundance have been conducted. Overall the preliminary results indicate that hypogeal fungi are not limiting at any of the Population Comparison Study sites where woylies have declined. However, more information regarding diet and feeding preferences is required. The aims, results so far and proposed future work are outlined in this report.

4.5.1. Introduction

An understanding of the biology and ecology of a threatened species is necessary for effective conservation and management (Chen *et al.,*. 1998) and the diagnosis of decline (Caughley 1994; Caughley and Gunn 1996; Peery *et al.,* 2004). Knowledge of the diet is an essential component of this understanding. The accessibility, abundance and suitability of resources, such as food, have been identified as a potential factor in the recent woylie declines (Wayne *et al.,* 2006). The woylie is mycophagous, excavating and ingesting large amounts of hypogeal (truffle-like) fungi and some epigeal fungi (i.e. mushrooms) throughout the year (Christensen, 1980; Davis, 2005; Garkaklis, 2001; Lamont *et al.*, 1985; Lee, 2003). However, no comprehensive studies have examined the temporal and spatial variation in diet and food resource availability.

The food resources component of the Woylie Conservation Research Project (WCRP) will be addressed by a collaborative PhD research project between Murdoch University and Department of Environment and Conservation (DEC). The aim of the PhD project is to determine if the dietary ecology of the woylie is related to current woylie population declines. Specifically the project aims to examine temporal and spatial variation in the diet of the woylie, examine changes in woylie diet in relation to population decline and to investigate food resource availability. In order to address these aims an analysis of dietary components will be conducted using scat samples and stomach contents. Food resource availability will be assessed by means of seasonal fungi and vegetation surveys. Data obtained from these studies will be interpreted in relation to climate change, land use history and the ecological role of woylies in ecosystem health and diversity. The PhD project commenced in January 2007 and is scheduled for completion early in 2010.

Preliminary studies were conducted by DEC researchers during 2006. The purpose of these studies was to develop the foundations for the collaborative PhD research project. In addition to collecting the scat and digesta samples for subsequent analysis, a pilot study was conducted to;

1. Survey hypogeal fungi at the WCRP Population Comparison Study (PCS) sites;
2. Conduct a preliminary assessment of whether there are any relationships between food resources and woylie abundances and population trends; and

3. Assess the feasibility and sampling requirements required to more rigorously test whether there are any significant differences in the hypogeal fungi food resources related to woylie abundances and trends.

The results of these studies are appended (Bougher 2006 and Robinson *et al.*. 2007) (Volume 2 Appendices 2 and 3). A summary of the key results from these studies as well as the results to date from the collaborative PhD project are detailed in this report.

4.5.2.1. Preliminary hypogeal fungi surveys

Preliminary surveys for hypogeous fungi were undertaken in June, August and September 2006 at five sites within the Upper Warren Region (Keninup, Balban, Warrup, Winnejup and Boyicup). A survey at Karakamia Wildlife Sanctuary, approximately 50 km east of Perth, was conducted on the 4th of October 2006. During each survey, three plots were established at each site – one lowerslope, midslope and upperslope location each. Plots were 20 m x 50 m with the long edge running along the slope. Advice regarding an appropriate plot size was sought from T. Lebel (Royal Botanic Gardens, Melbourne) prior to the study. Hypogeous sporocarps were collected by raking the litter and organic soil layer with a four-pronged Canterbury hoe to a depth of 10-15 cm. Each plot was raked for the equivalent of 100 person minutes. The aim was to cover approximately 25-40% of the total plot area, with an effort made to distribute the search evenly across each plot.

All fungal sporocarps were collected and assigned a collection number. Sporocarps were described, assigned a species number and photographed in the laboratory. Collections were then air dried and weighed. The “reference type collection” for each species was then microscopically examined, photographed and where possible identified.

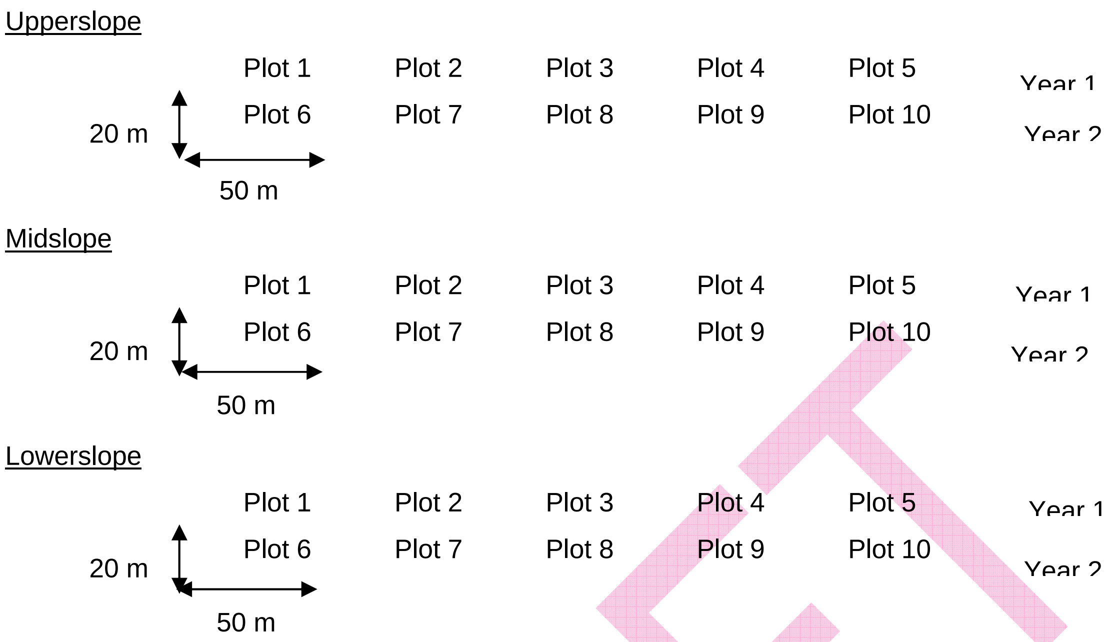
Vegetation structure and floristics data was collected during the preliminary assessment, but has not yet been analysed. Tree basal area, overstorey, midstorey and lowerstorey species cover and strata heights were recorded in each plot. All plots were assigned a vegetation health score and *Phytophthora cinnamomi* presence score. Soil colour and type were also noted along with the amount of litter in each plot. To aid in determining relationships between hypogeous fungal diversity and abundance, leaf litter and soil moisture data was collected during the September fungal survey.

For a detailed description of the methods used in these preliminary surveys please refer to the reports contained within Volume 2, Appendices 2 and 3

4.5.2.2. PhD project fungi surveys

The survey design for the PhD project builds on the design used for the preliminary surveys (Bougher, 2006; Robinson *et al.,* 2007). The same six woylie PCS sites will be surveyed during the PhD project, Keninup, Balban, Warrup, Winnejup, Boyicup and Karakamia Wildlife Sanctuary. As with the preliminary surveys, during each survey period, three plots will be surveyed in relation to topography – one upperslope, midslope and lowerslope plot at each site (Figure 4.5.1). Each plot will be 20 m x 50 m with the longest edge running across slope. A randomised-block design for repeat sampling within each topographic grouping is used to account for possible confounding by environmental factors (Figure 4.5.1). Over a two year period there will be a survey within each season, with extra surveys during winter as a greater number of fungi fruit at this time. A survey will be conducted in Winter 2007 (two surveys), Spring 2007, Summer 2007/08, Autumn 2008, Winter 2008 (two surveys), Spring 2008, Summer 2008/09 and Autumn 2009. Therefore, a total of 10 plots will be surveyed at each topographic position during 10 survey periods at each site. Each of the ten plots within each topographic position will be randomly assigned to a seasonal survey.

Fungi survey plots will be situated within 5 km of the population monitoring trapping grid at each site and will not be situated in recently burnt (< 6 months) areas, or near well used roads.



Note: Plots 1-10 within each topographic grouping will be randomly assigned to a seasonal survey.

Figure 4.5.1. Design of PhD research project fungi survey at each site.

Collection of hypogeal fungi sporocarps

Plots will be sampled using a time census method, developed by Claridge *et al.,* (2000). Based on the results of Robinson *et al.,* (2007) a 100 person minute sampling time will be used to survey each plot. Hypogeal fungi will be collected using a four-pronged hoe to rake the litter and soil layer to a depth of approximately 15 cm. Disturbed soil will be replaced immediately after the survey. All sporocarps will be collected. It is anticipated that the collections from the preliminary surveys and the PhD program will be joined and submitted to the Western Australian Museum upon completion of the project.

Processing and identification of sporocarps

In the laboratory, each sporocarp collection will be identified to species or morphospecies. Specimens will then be dried for 4-5 days in a drying cupboard or food dehydrator at 40°C. A dry weight for each specimen will be recorded. To confirm identifications a sample will be taken from dried specimens using a razor blade to cut a thin section of gleba and mounted on a glass slide. Specimens will be stained with KOH and in some cases Melzer's reagent. The spores will be examined under x1000 magnification using a compound microscope. A photograph will be taken to be used as a reference for the identification of fungal spores in the woylie faecal samples.

Surveying of epigeal fungi sporocarps

Bougher (2006) recommended that epigeal fungi are included in future surveys in response to evidence that mammals at Karakamia have been observed feeding on epigeal fungi. As a result epigeal fungi will also be surveyed as part of the PhD project. Each plot will be surveyed thoroughly for epigeal fungi, prior to commencement of the hypogeal sporocarp searches. All epigeal species present within the plot will be identified (where possible) and recorded. Due to the large number of epigeal species present within the plots, the numbers of species rather than fruiting bodies will be recorded.

The date, plot location, associated vascular plants, time since last burn, litter layer depth and soil characteristics (soil type, soil colour and soil moisture content) will also be recorded for each plot.

Robinson *et al.* (2007) recommended that portable weather stations be installed at each site in order to analyse relationships between climatic conditions and fungal species diversity and richness, as rainfall patterns in the Upper Warren region appear to be variable and highly localized. Rainfall stations have now been installed at each of the five population comparison study sites within the Upper Warren Region.

At the time of this report, two surveys to assess the availability of epigeal and hypogeal fungal sporocarps (fruiting bodies) at each of the six PCS sites, Karakamia, Keninup, Balban, Warrup, Winnejup and Boyicup have been conducted as part of the PhD research project. The results from these two surveys are included in this report.

4.5.3. Results

4.5.3.1. Preliminary hypogeal fungi surveys

During the three surveys within the Upper Warren Region, 388 collections comprising 826 sporocarps of hypogeous fungi were made. Thirty-four species of fungi were recognized. Thirty-one species were Basidiomycetes; two were Glomeromycetes (*Endogone* spp.) and one was an Ascomycete (*Hydnoplicata convoluta*). Seven were identified to species level and 15 were identified to genus only. Full collection details are provided in Robinson *et al.* (2007) (Volume 2 Appendix 2).

Five species were represented among 11 collections from Karakamia Sanctuary during the survey in October 2006, three Basidiomycetes and two Zygomycetes. Two species, *Cytangium sessile* and *Dermocybe globuliformis* were collected from both Karakamia Sanctuary and the Upper Warren Region. Identifications, descriptive details, and images of the specimens are provided in Appendix 3 (Volume 2).

The results of the preliminary fungi surveys showed that the highest species diversity and abundance was recorded in August. Overall, species diversity was similar between the sites. However, species composition varied considerably for each of the sites, with only a few recorded species common to all sites. Sporocarp abundance varied between sites. Generally, abundance was found to be higher at sites with higher rainfall.

The lowest overall number of sporocarps was collected from upperslope sites. The only exception was Boyicup, where the most sporocarps were recorded from the upperslope site. Correspondingly, the upperslope sites also recorded the lowest overall total dry weight of sporocarps.

The total number and biomass of sporocarps tended to be inversely related to woylie abundance, which suggests that hypogeal fungi (and presumably food resources) were available at sites where woylies have declined, Analysis of the data from the preliminary work relating to vegetation structure and floristics and woylie nest and digging densities remain to be completed (Wayne unpublished data).

The full reports for the preliminary fungi surveys are included as Appendix 2 and Appendix 3 (Volume 2).

4.5.3.2. PhD project fungi surveys

Epigeal Fungi

During the two surveys in June and August 2007, 88 species were recorded from Boyicup, 77 species from Warrup, 52 species from Keninup, 64 species from Balban, 65 species from Winnejup and 59 species from Karakamia. Only some of these species are likely to be eaten by woylies. Although this will not be known until the results of the dietary scat analysis are available (see Section 4.5.5. Future Work).

Hypogeal Fungi

To date, 595 sporocarps have been collected; totalling 62.8 grams (dry weight). Up to 29 hypogeal fungi species from the first survey (Winter 1) and 27 hypogeal fungi species from the second survey (Winter 2) have been recorded. Approximately 45 species in total have so far been recorded. It is likely that a number of new species have already been discovered. Further taxonomic work is required to confirm identifications. In addition, reference slides are being produced from these samples to aid with the faecal analysis (see Section 4.5.5. Future Work).

A greater number of sporocarps were collected during the August survey (Winter 2), with 345 sporocarps collected compared with 250 sporocarps collected in June 2007 (Winter 1) (Figure 4.5.2). Soil moisture was generally higher during the second (Winter 2) survey (Figure 4.5.3). The highest number and dry weight of sporocarps was collected from plots at Boyicup in the Upper Warren Region (Figures 4.5.2 and 4.5.4). Soil moisture content was also the highest at Boyicup during both surveys (Figure 4.5.3). Conversely, the lowest number of sporocarps and total dry weight was recorded from Karakamia Sanctuary (Figures 4.5.2 and 4.5.4). A similar result was observed during the preliminary surveys (Volume 2 Appendices 2 and 3).

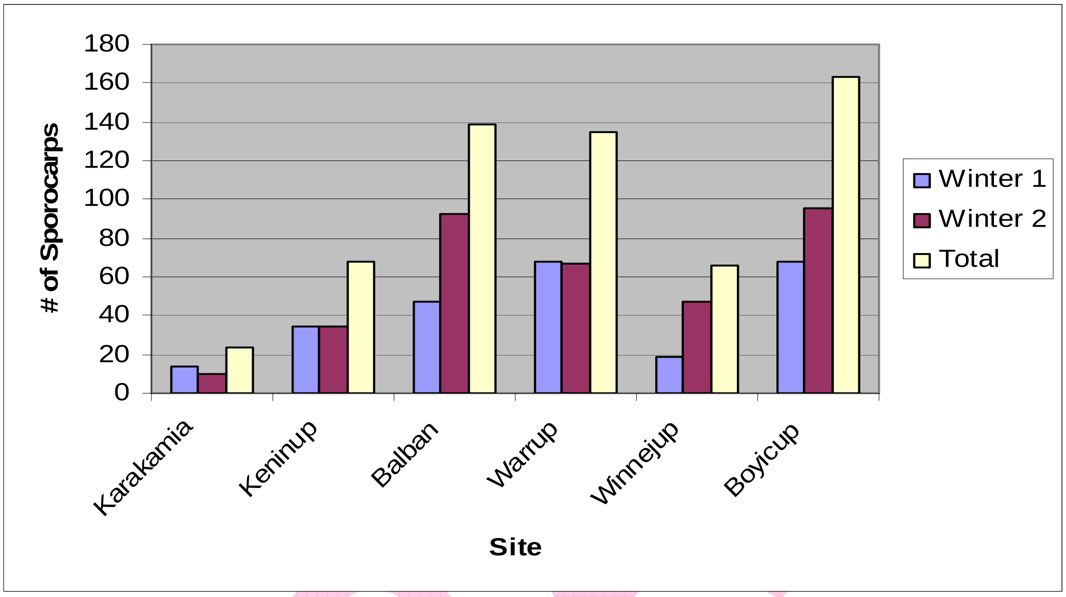


Figure 4.5.2. Number of hypogeal sporocarps collected during the two surveys at the six woylie PCS sites.

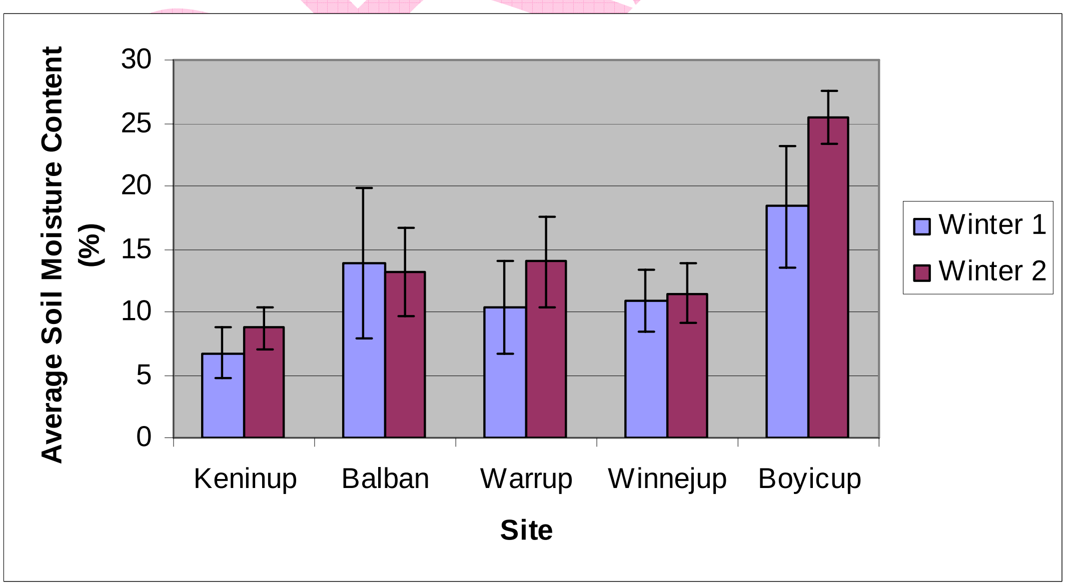


Figure 4.5.3. Average soil moisture content (%) recorded during the two winter surveys at each of the five woylie PCS sites in the Upper Warren.

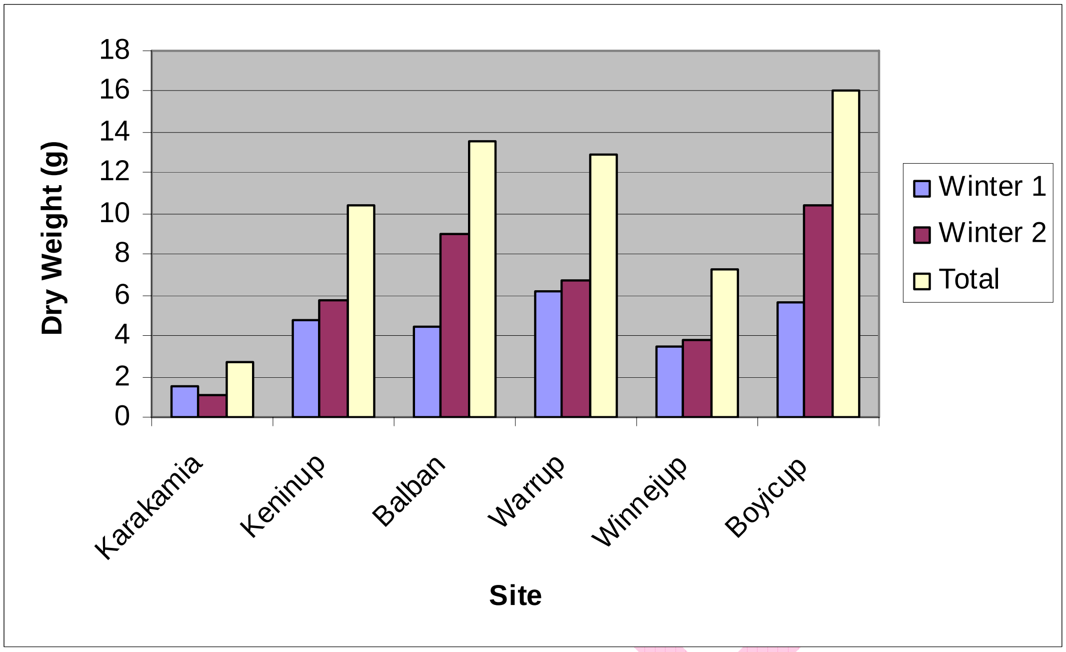


Figure 4.5.4. Total dry weight (grams) of sporocarps recorded during the two winter surveys at each of the six woylie PCS sites.

The majority of sporocarps collected to date during the PhD project have been from upperslope plots, followed by the midslope and lowerslope plots (Figure 4.5.5). This is in contrast to the results of the preliminary surveys where the fewest sporocarp collections were made from upperslope plots (Volume 2 Appendix 2). However, the relationship between the number of sporocarps collected and topographic position varies considerably at each site (Figure 4.5.6).

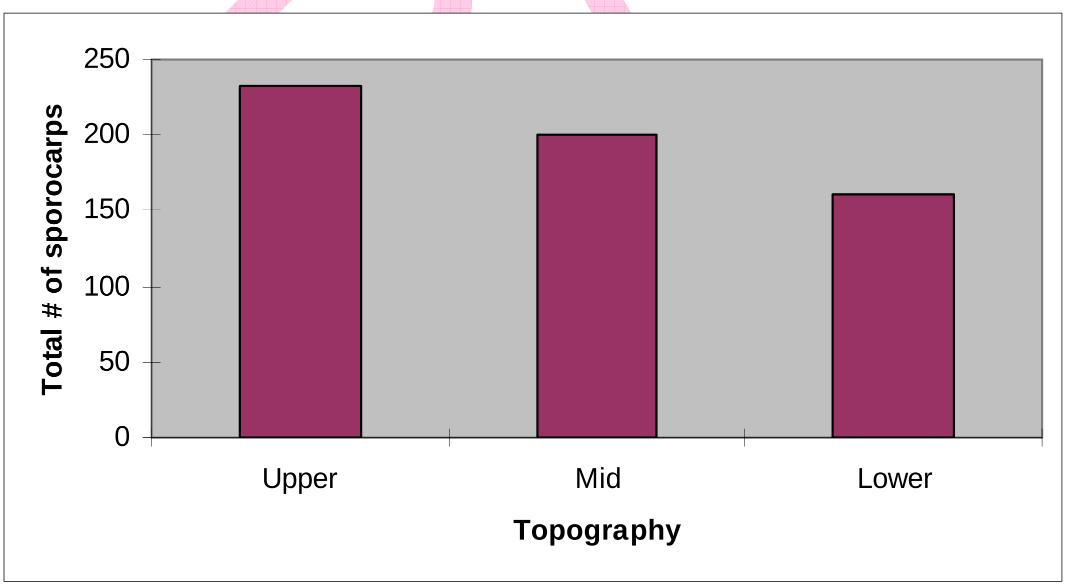


Figure 4.5.5. Total number of hypogeal sporocarps collected within each topographic grouping.

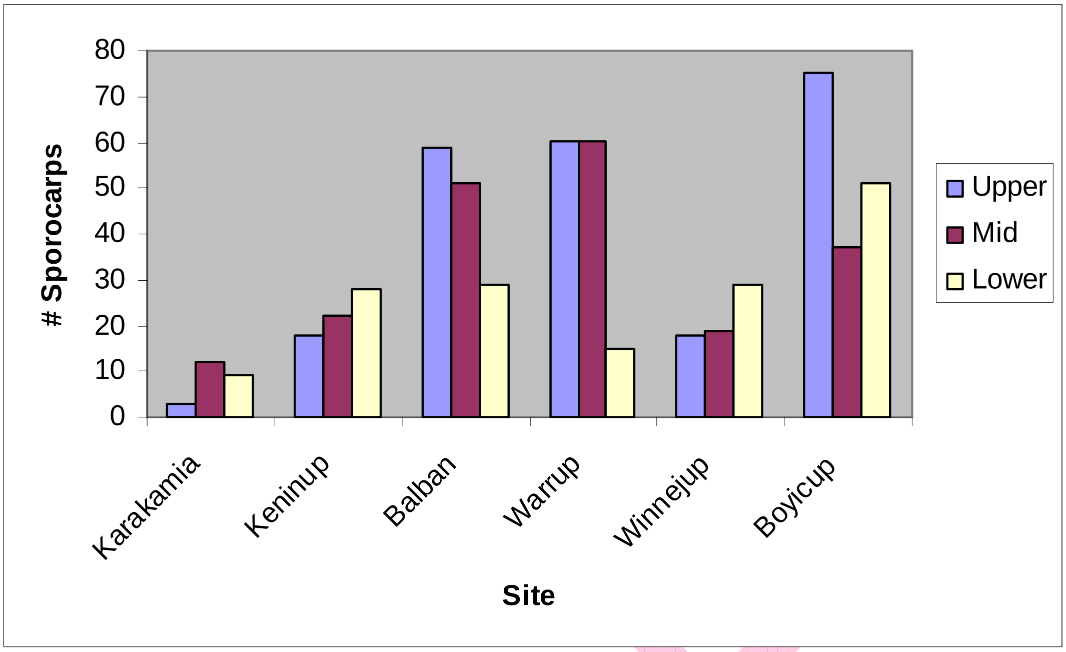


Figure 4.5.6. Number of hypogeal sporocarps collected within each topographic grouping at the six woylie PCS sites.

4.5.4. Discussion

The hypogeal habit, ephemeral nature and patchy distribution of sequestrate fungal sporocarps, means that their availability is difficult to measure (Bougher and Lebel, 2001). Previous studies have had difficulties due to a small number of collections or because a large number of plots was required. However, the results of the preliminary surveys and the two surveys conducted as part of the PhD project indicate that the methodology has so far been successful, with a large number of collections.

Previous studies have found seasonal differences in the abundance of fungi, where some species demonstrate peaks in abundance during wetter periods (Abell *et al.,* 2006; Claridge *et al.,*. 1993). While the studies conducted so far, as part of the WCRP have only involved sampling in winter and spring, it seems likely that there will be a significant seasonal trend in the number of sporocarps recorded. More sporocarp collections were made during August for both the PhD project and during the preliminary survey. This is likely to be the result of more appropriate conditions for fruiting, as soil moisture content was also found to be higher during the August surveys. Previous studies have also shown a relationship between soil moisture levels and fruiting, where those species with sporocarps of high moisture content fruited when soil moisture was also high (Johnson, 1994). A number of hypogeal species recorded during the Winter 1 survey were not recorded during the Winter 2 survey and vice versa.

Further research regarding dietary preferences of woylies is required before the consequences of seasonal variation in the number and abundance of fungi species for woylies can be determined. If the availability of hypogeal sporocarps is limiting at certain times of the year and woylies are reliant on other food sources, this may influence woylie survival and reproductive output.

No real trend with regard to topographic position and the number of collections made has been observed at this stage. However, this may become more prominent during the drier months, where lowerslope sites are more likely to retain soil moisture.

During the preliminary surveys and the two surveys for the PhD project, the highest number of sporocarp collections was made at sites where woylies are not present in any great numbers. Conversely, very few collections were made where woylies are present in high densities. Robinson *et al.*. (2007) suggested that this result could be related to the foraging activities of woylies, whereby sporocarps are more difficult to unearth where researchers are in competition with woylies. While this is likely to be true, the preliminary results also indicate that hypogeal fungi are not limiting at any of the population comparison sites where woylies have declined. However, more information regarding diet and feeding preferences is required.

4.5.5. Future work

The collaborative PhD research project between Murdoch University and DEC is still in its early stages (i.e. started January 2006). Future work will involve continuing the fungi availability surveys and commencing the woylie dietary analysis.

Future work for DEC Science should also include an analysis of the vegetation structure and floristics data collected during the preliminary surveys and the completion of vegetation surveys within each of the PCS trapping grids. This data will be related to both food and woylie abundance.

Woylie Dietary Analysis

An analysis of dietary components will be conducted using faecal samples collected during population monitoring and opportunistically from the stomach contents of recovered woylie bodies. This approach has the advantage of providing a large quantity of information on the diet of the woylie while using non-invasive procedures.

A large amount of faecal material has been collected (and will continue to be collected) from woylies during trapping programmes run by the Department of Environment and Conservation, Australian Wildlife Conservancy and the South Australian Department of Environment and Heritage. Faecal material for dietary analysis has been collected from a number of locations within the Upper Warren region, Karakamia Wildlife Sanctuary, Tutanning Nature Reserve, Dryandra Woodland, Batalling State Forest and Venus Bay Conservation Park (SA).

Each faecal sample collected has been labelled with a unique code and information on sex, date of capture, location of capture and the health and reproductive condition of the individual has been recorded. The faecal analysis will be based upon the methodology used by Tory *et al.* (1997) and Green *et al.* (1999). Briefly, this will involve filtering the faecal material into a coarse and fine fraction and then examining a series of sub-samples of each fraction under a compound microscope. Food items will be identified to the lowest grouping possible and the relative percentage of each dietary category calculated by examining a set number of fields of view.

To investigate spatial variation in the diet of woylies, a selection of faecal samples from corresponding seasons will be assessed from each of the locations mentioned earlier. Temporal variation will be assessed by investigating the different components of woylie diets and examining any differences in the relative proportion of dietary components between seasons, over a two year period, from woylie populations at Karakamia Sanctuary and the Tone Perup Nature Reserve/Greater Kingston National Park, where sampling has been much more frequent. In addition, dietary comparisons will be made during the various stages of reproduction.

To examine changes in woylie diet in relation to population decline, three sets of woylie faecal samples will be analysed. Firstly, the dietary composition and proportions of dietary items will be compared for woylies in populations of high (Karakamia Sanctuary), moderate (Warrup and Keninup) and low (Boyicup and Winnejup) densities. Secondly, the composition and proportions of dietary items will be analysed for woylies from the Balban site, during the transition from a moderate density population to a low density population. Thirdly, the composition and proportions of dietary components will be compared for woylies pre decline (pre 2001) and post decline. Pre-decline samples are available from other studies conducted in the Upper Warren Region and Batalling before woylie populations started to decline in number (prior to 2001).

To compliment the results of the faecal sample analysis, stomach contents collected opportunistically from deceased woylies will be analysed. Stomach contents are generally accepted to be more reliable as the dietary items are less digested than those found in faecal samples. This will then provide a comparison for the faecal analysis, and allow a determination of the level of accuracy with which the faecal results may be interpreted.

As well as forming the basis for a PhD thesis, it is expected that a number of publications of articles in scientific journals will result from this research.

4.5.6. Conclusion

The results of the fungi availability surveys conducted to date indicate that food resources may not be limiting at any of the PCS sites where woylie numbers have substantially declined (Boyicup and Winnejup). In fact, fungal sporocarps are in lower abundance at Karakamia Wildlife Sanctuary and Keninup where woylie numbers are the highest. However, it is premature to draw conclusions and caution is required when interpreting the results because of the preliminary nature of the work. For example, it is imprudent to assume that all of the fungal species recorded are suitable to be consumed by woylies. More information regarding diet and feeding preferences will be determined once the faecal analysis has been conducted. Both food resource availability and dietary analysis need to be assessed to identify relationships between diet and seasonal changes in the abundance of food resources. It seems likely, however, that the number and abundance of fungi species and other dietary items will vary seasonally. As a consequence, there may be times of the year when preferred food resources are limiting and this may influence woylie survival and reproductive output.

Information obtained during the PhD research project will not only assist with the diagnosis of current woylie declines, but will also enable a better understanding of long-term conservation requirements. An understanding of when food resources may be limiting and temporal and spatial variations in sporocarp production will assist future recovery programmes, including re-introductions and translocations.

4.5.7. References

Abell, S. E., P. A. Gadek, C. A. Pearce, and B. C. Congdon. 2006. Seasonal resource availability and use by an endangered tropical mycophagous marsupial. Biological Conservation 132:533-540.

Bougher, N. L. 2006. Identity and taxonomy of truffle fungi from an initial survey at Karakamia Wildlife Sanctuary. Department of Environment and Conservation.

Bougher, N. L., and T. Lebel. 2001. Sequestrate (Truffle-like) fungi of Australia and New Zealand. Australian Systematic Botany 14:439-484.

Caughley, G. 1994. Directions in conservation biology. Journal of Animal Ecology 63:215-244.

Caughley, G. C., and A. Gunn 1996. Conservation Biology in Theory and Practice. Blackwell Science, Cambridge.

Chen, X., C. R. Dickman, and M. B. Thompson. 1998. Diet of the mulgara, *Dasycercus cristicauda* (Marsupialia : Dasyuridae), in the Simpson Desert, central Australia. Wildlife Research 25:233-242.

Christensen, P. S. 1980. The biology of *Bettongia penicillata* Gray, 1837, and *Macropus eugenii* (Desmarest, 1817) in relation to fire in F. D. o. W. Australia, editor. Forests Department of Western Australia.

Claridge, A. W., A. Robinson, M. T. Tanton, and R. B. Cunningham. 1993. Seasonal production of hypogeal fungal sporocarps in a mixed-species Eucalypt forest stand in south-eastern Australia. Australian Journal of Botany 41:145-167.

Claridge, A. W., S. J. Cork, and J. M. Trappe. 2000. Diversity and habitat relationships of hypogeous fungi.I. Study design, sampling techniques and general survey results. Biodiversity and Conservation 9:151-173.

Davis, J. E. 2005. Mycophagy in the brush-tail possum (*Trichosurus vulpecula*) leading to dietary overlap with the woylie (*Bettongia penicillata*) in Dryandra Woodland, Western Australia. Murdoch University, Perth.

Garkaklis, M. J. 2001. Digging by the woylie *Bettongia penicillata* (Marsupialia) and its effects upon soil and landscape characteristics in a Western Australian woodland. Murdoch University.

Green, K., M. K. Tory, A. T. Mitchell, P. Tennant, and T. W. May. 1999. The diet of the long-footed potoroo (*Potorous longipes*). Australian Journal of Ecology 24:151-156.

Johnson, C. N. 1994. Fruiting of hypogeous fungi in dry sclerophyll forest in Tasmania, Australia: seasonal variation and annual production. Mycological Research 98:1173-1182.

Lamont, B. B., C. S. Ralph, and P. S. Christensen. 1985. Mycophagous marsupials as dispersal agents for ectomycorrhizal fungi on *Eucalyptus calophylla* and *Gastrolobium bilobum*. New Phytologist 101:651-656.

Lee, J. 2003. The importance of hypogeous fungi in the diet of the re-introduced brush-tailed bettong (*Bettongia penicillata*) at Venus Bay Conservation Park, South Australia. School of Earth and Environmental Sciences. Adelaide University, Adelaide.

Peery, M. Z., S. R. Beissinger, S. H. Newman, E. B. Burkett, and T. D. Williams. 2004. Applying the declining population paradigm: Diagnosing causes of poor reproduction in the Marbled Murrelet. Conservation Biology 18:1088-1098.

Robinson, R., J. Fielder, M. Maxwell, N. L. Bougher, W. Sicard, and A. Wayne. 2007. Woylie conservation research project: Preliminary survey of hypogeous fungi in the Upper Warren region in D. o. E. a. Conservation, editor.

Tory, M. K., T. W. May, P. J. Keane, and A. F. Bennett. 1997. Mycophagy in small mammals: A comparison of the occurrence and diversity of hypogeal fungi in the diet of the long-nosed potoroo *Potorous tridactylus* and the bush rat *Rattus fuscipes* from southwestern Victoria, Australia. Australian Journal of Ecology 22:460-470.

Wayne, A., I. Wilson, J. Northin, B. Barton, J. Gillard, K. Morris, P. Orell, and J. Richardson. 2006. Situation report and project proposal: identifying the cause(s) for the recent declines of woylies in south-western Australia. in D. o. C. a. L. Management, editor. A report to the Department of Conservation and Land Management Corporate Executive. Perth, Western Australia.

4.6. PCS expansion – Inclusions of woylie data from external programs

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Abstract

Additional populations were specifically sampled to complement and expand the population comparisons that have focused on the Upper Warren region and Karakamia. This was in response to the early findings from some of the woylie conservation research project disease investigations and concurrent woylie declines in South Australia. The additional sites included Batalling forest, Dryandra and Tutanning Nature Reserves in Western Australia, and Venus Bay Peninsula Conservation Park, Venus Bay Island A and St Peter's Island in South Australia. The additional samples were opportunistically collected in collaboration with the workers responsible for the pre-existing programs at these sites. Samples were used to assist specific disease investigations, conservation genetics, dietary analysis, and necropsy and pathology samples from dead woylies. Details of the additional sites samples are briefly summarized here. The results from the additional sampling sites are addressed within the specific report sections for which the samples were principally collected.

4.6.1. Introduction

The Woylie Conservation Research Project (WCRP) has primarily focussed on the woylie populations within the Upper Warren region and Karakamia Wildlife Sanctuary (i.e. Population Comparison Study (PCS) sites and Upper Warren Fauna Monitoring transects). The reasons for this focus are provided in the Introduction of this report (Chapter 1) and the Science Project Proposal (SPP 2007/02). Other woylie populations in Western Australia and South Australia however, offered additional scope for comparison ranging from apparently 'healthy' and 'stable' populations to those experiencing a decline, isolated island populations and natural and translocated populations. The targeted inclusion of additional populations in this project was made in response to disease findings of distinct differences between the Karakamia and Upper Warren - namely the absence of *Toxoplasma* and *Trypanosoma* in the former. Within this context, reports received in late 2006 of characteristically similar declines from Venus Bay Peninsula (VBP), South Australia were also considered as potentially important and instructive in helping to identify possible causes of the seemingly evermore extensive woylie declines.

The inclusion of additional sites relied on existing trapping programs being undertaken by workers independent of the WCRP. These additional populations included Batalling forest, Dryandra and Tutanning Nature Reserves in Western Australia, and VBP Conservation Park, Venus Bay Island A (VBA) and St Peter's Island in South Australia. The inclusion of these sites has been predominantly limited to the collection of samples specifically relevant to disease and conservation genetics, and opportunistically scats for woylie dietary analysis as part of the PhD research program into woylie food resources by Kerry Rodda.

While the WCRP provided the protocols, equipment, support and guidance, the collection of the additional 'external-program' samples depended on the co-operation and efforts of the workers responsible for these opportunities. Specialist assistance in phlebotomy was provided by qualified vets and WCRP collaborators, Carlo Pacioni and Sabrina Trocini at Dryandra and Tutanning (i.e. where required). DEC employees Christina Gilbert (qualified nurse) and Fiona Kirkpatrick trained and assisted with disease sampling in the Upper Warren prior to taking similar samples in Batalling. Qualified vets, Monarto Zoo and DEHSA staff conducted the sampling in South Australia.

Through direct collaboration and co-operation, the necropsy and pathology of woylie mortalities associated with the mesopredator research in Dryandra and Tutanning (Nicky Marlow *et al.*) were also incorporated into the protocols associated with WCRP. Sick, injured or recently dead woylies encountered during other woylie-associated activities that were reported to the WCRP were also investigated by the WCRP.

A brief summary is provided for the additional sites sampled by collaborators in association with the WCRP:

4.6.2. Other WA populations

4.6.2.1. Batalling forest

Principal Collaborator: Fiona Kirkpatrick, DEC Collie

Site history: Woylies were translocated from Perup to the Batalling area in 1983. A healthy and expanding population was recorded in 1993 (Start *et al.*, 1995). Between 2002 and 2007 a decline of 97% has been observed in the woylie population.

Reason for inclusion: The cause(s) of this decline are unknown making the Batalling population of interest to this study.

Data/Sample collection: Data and samples were collected from Varis Road and Steed Road transects on 22nd -25th November 2006 and 27th – 30th November 2007 by Fiona Kirkpatrick, Christina Gilbert and DEC Collie District colleagues

4.6.2.2. Dryandra Nature Reserve

Principal Collaborator: Nicky Marlow, DEC Science Division, Woodvale

Site history: Dryandra supports one of the three last remaining natural (indigenous) woylie populations. The woylie capture rate has declined by 93% (2000 – 2006).

Reason for inclusion: The cause(s) of this decline are unknown making the Dryandra population of interest to this study.

Data/Sample collection: Data and samples were collected on 21st-24th November 2006 by Nicky Marlow and the meso-predator research project team. *Western Shield* trapping data is available but not yet analysed. Pathology samples were collected from dead radio-collared woylies sent to Murdoch University for necropsy to assist in the determination of factors associated with the mortality.

4.6.2.3. Tutanning Nature Reserve

Principal Collaborator: Nicky Marlow, DEC Science Division, Woodvale

Site history: Tutanning supports one of the three last remaining natural (indigenous) woylie populations. It has remained at low densities for about a decade, with no apparent indications of recent decline.

Reason for inclusion: An apparently stable, but low density population, provides a powerful comparison to declined populations such as Dryandra and Upper Warren.

Data/Sample collection: Data and samples were collected on 28th November 2006 by Nicky Marlow and the meso-predator research project team. Additional data and samples were collected by Peter Orell and Christine Freegard on 10th-12 April 2007 during tammar translocation monitoring. *Western Shield* trapping data is available but not yet analysed. Pathology samples were collected from dead radio-collared woylies sent to Murdoch University for necropsy to assist in the determination of factors associated with the mortality.

4.6.3. South Australian Populations

4.6.3.1. Venus Bay Peninsula/Monarto Zoo

Principal Collaborators: Jason van Weenan and David Armstrong (South Australian Department of Environment and Heritage) and Ian Smith (Monarto Zoo, South Australia).

Site history: VBP was the first unfenced reintroduction of woylies to the mainland in South Australia, occurring in 1994. By 2002, the woylie population had increased and expanded to the northern section of the peninsula. A steady decline in the southern half of the peninsula was observed from early 2005. This appeared to gradually extend into the northern half, corresponding with the way in which the population initially expanded north up the peninsula, culminating in a sudden massive decline of more than 90% between March and July 2006. A total of 53 woylies from VBP were sent to Monarto Zoo for captive holding during the woylie decline to insure against population extinction.

Possible cause(s) of decline: A combination of limited food resources, cat predation and severe weather events has more recently been hypothesised as the cause for the extreme decline seen in this population (Section 4.7 VBP summary). Reason for inclusion: The decline observed in the VBP population was of interest to this study due to a simultaneous decline in isolated Western Australian and South Australian woylie populations. Initially the cause of the declines were unknown. Subsequent hypothesis of the cause(s) remain untested/unsubstantiated.

Data/Sample collection: Monarto Zoo staff collected data and samples from the captive woylies in December 2006 and May 2007.

4.6.3.2. Venus Bay Island A

Principal Collaborators: Jason van Weenan and David Armstrong (South Australian Department of Environment and Heritage)

Site history: This translocated island population is descended from seven captive bred animals introduced in 1980. The island of 17 hectares supports only a small woylie population. Since an initial boom-crash cycle the population has stabilised at between 25 and 35 individuals (Start *et al.*, 1995). Monitoring conducted in December 2006 revealed half the animals were in poor condition (D.Armstrong, DEH, SA, pers. comm.). On the latest monitoring trip in June 2007, 22 animals were caught, 16 of which were new animals. The animals appeared to be in good condition.

Possible cause(s) of decline: The poor body condition and lack of adult survival is thought to have been caused by the harsh summer drought experienced in 2006/2007 and a subsequent lack of resources (D. Armstrong, DEHSA, pers. comm.).

Reason for inclusion: VBA was of interest to this study as an isolated island reference population with no introduced predators.

Data/sample collection: On 26th June 2007 data and samples were collected by David Armstrong.

4.6.3.3. St Peter's Island

Principal Collaborator: Jason van Weenan (South Australian Department of Environment and Heritage)

Site history: This translocated island population is descended from a total of 113 Woylies released in 1989 from captive populations held in Canberra and Monarto. In 1993 the population was recorded as being well established and growing quickly (Start *et al.*, 1995). The island is 3 439 hectares in size and no decline has been observed.

Reason for inclusion: This island population was of interest to this study as a reference population that had not shown decline in numbers, has no introduced predators and is an isolated population.

Data/sample collection: Data and samples were collected from 78 animals captured on 9th-11th June 2007 by Jason van Weenan.

4.6.3.4. Other woylie individuals/populations

Liaison with workers closely associated with other woylie populations has included Scotia Wildlife Sanctuary (AWC, NSW), Paruna Wildlife Sanctuary (AWC, WA), St Johns forest (DEC, WA), and Julimar (DEC, WA). Liaison with individuals or organisations with private colonies and/or are wildlife carers have included establishments in Northcliffe, Yelverton, Boyup Brook, Kalamunda, Wellard, Roleystone and Mt Helena. Liaison has also occurred with individuals investigating possible repeated woylie sightings near Fitzgerald National Park (SCNRM, WA).

Liaison has generally involved i) encouraging strategies to minimise the potential spread of possible agents of decline, ii) close surveillance of woylies for signs of population decline and/or individuals displaying symptoms or poor health, iii) recovery of any dead or dying animals, and iv) reporting any cases of sick or dead woylies when they have been encountered.

A necropsy was conducted on one of three woylies that died at a private captive colony in Wellard, Perth. The three animals died in November 2006 without a known cause. The details of this case are provided in the pathology section of the report (Section 5.4). There were three incidences of metabolic bone disease (two euthanased, one survived) in hand-reared woylies provided by a wildlife carer to Yelverton Eco Retreat. Details of these cases are provided in the Clinical section of the report (Section 5.3).

4.6.4. References

Start, T., Burbidge, A. and Armstrong, D. 1995. Woylie Recovery Plan (2nd Edition). Western Australian Wildlife Management Program No. 16, Department of Conservation and Land Management.

4.7. Venus Bay Peninsula (SA) woylie summary

David Armstrong

Department of Environment and Heritage, South Australia

4.7.1. When and where the woylie decline was first noticed and how quickly it spread.

Monitoring showed a steady decline in the lower half of the peninsula from early 2005. This appeared to gradually extend into the northern half, and a sudden massive decline occurred between March/April 2006 and early July 2006.

Three types of monitoring are carried out approximately quarterly

- 12 km spotlight transect down the peninsula, commenced in 1992, with some variation in method and frequency over that time. In particular the introduction of an oat trail to assist in counting woylies from January 2002. Animals observed are collated in 500 m intervals.
- 4.5 km spotlight transect along an oat trail around the first paddock inside the fence, started in January 2005. This was principally intended to assess bilby numbers, which were and still are concentrated in this paddock.
- Sample trapping started late 2005. Four sets of ten traps at 100 m intervals. Two trap sets in the upper and two in the lower peninsula.

The 12 km spotlight transect figures show a steady decline in woylie numbers in the lower half of the peninsula from a peak in early 2005 (Figure 4.7.1). This is supported by the limited trapping figures (Table 4.7.1) available from November 2005 onward, which show a sudden decline in March 2006 for the southern half of the peninsula. However, although the woylie count in the top paddock transect (Table 4.7.2) had peaked at 345 in June 2005, they were still over 100 in March 2006. When the number dropped to 15 by early July 2006, it was clear something dramatic had happened. The 12 km transect and sample trapping were completed as soon as possible following the initial concern created by the shorter transect results and both confirmed a sudden decline in woylie numbers in the northern half of the peninsula also.

The drop in numbers in the lower peninsula first, followed later by a drop in the northern half, corresponds with the way in which the population initially expanded up the peninsula from the 1994 release sites in the south. It was only early in 2002 that woylies were first seen in the northern half above the blown out sand dune and cleared paddock. It seemed as though they were reluctant to cross the open area for some time, until forced to by competition for food and nesting territories. The numbers in the northern half then increased rapidly over about the first year, until in 2003-05 it was common to see woylies feeding in the open up to 500 metres from any sheltered areas of scrub. On a number of occasions woylies were seen emerging from or retreating down rabbit or bilby burrows. Both these behaviours are extremely unusual behaviour for woylies, and are attributed to high population stress.

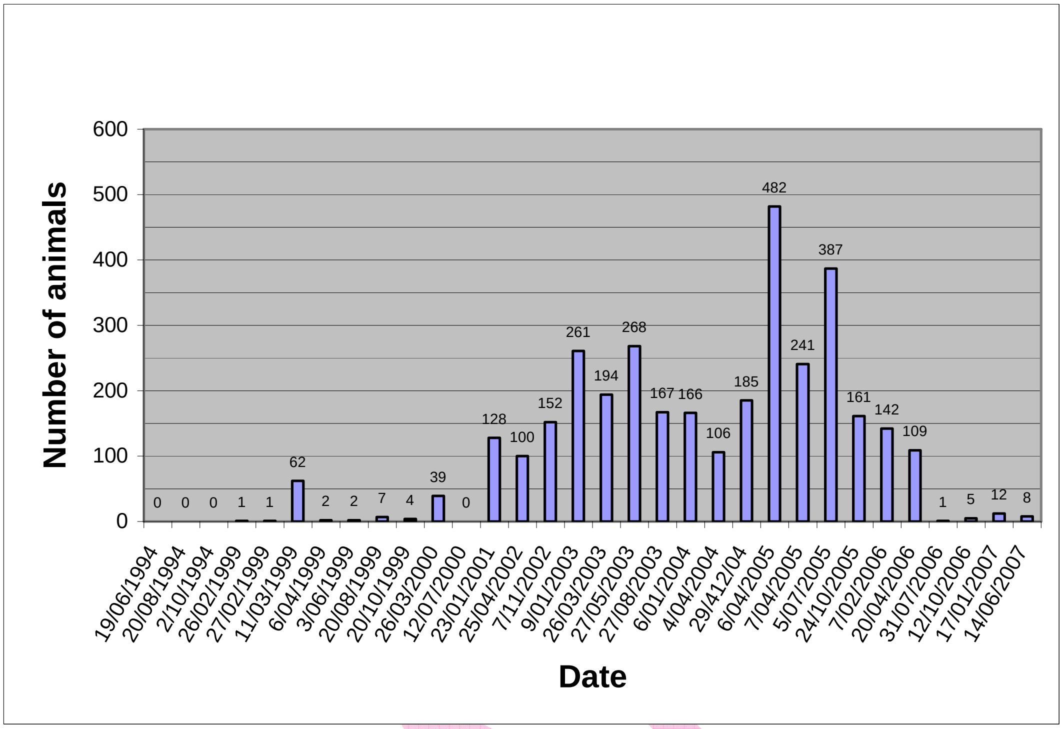

Figure 4.7.1. Venus Bay woylie population trends from 12 km spotlight transect.

Table 4.7.1. Woylie captures from ten traps at 100 m intervals along vehicle tracks, four traplines (A,B, C+D).

DATE	A	B	C	D	Total
22/11/05	5	4	5	3	17
2/3/06	7	2	0	2	11
4/7/06	1	2	0	0	3
26/1/07	1	2	0	5	8
17/5/07	4	1	4	6	15
5/9/07	2	2	2	4	10

20 woylies were re-released at the other end of the paddock containing trapline A (approx 2.0 km away) on 20/12/06. A further 10 woylies were re-released in the area of trapline A on 4/6/07.

Table 4.7.2. Results of 4.5 km spotlight transect along oat trail around perimeter track of top paddock inside Venus Bay Conservation Park fenced area.

DATE	BILBIES	WOYLIES	RABBITS
31/1/05	21	137	33
20/3/05	19	124	38
8/6/05	33	345	15
12/9/05	37	159	18
16/1/06	28	166	11
15/3/06 (a)	20	118	3
15/3/06 (b)	26	157	2
4/7/06	20	15	21
23/7/06 (a)	18	0	20
23/7/06 (b)	21	0	5
12/10/06	5	0	12
23/11/06	11	0	19
30/1/07	32	7	43
20/3/07	22	2	6
16/5/07	24	5	12
25/8/07	3	7	11

20 woylies were re-released in this area on 20/12/06 followed by another 10 woylies on 4/6/07.

4.7.2. Estimated level of decline in woylie population

The number of woylies observed on both spotlight transects has dropped significantly. The drop on the 12 km transect, from a peak of over 300 two years ago, to 12 and eight for the last two counts, and from over 300 for the 4.5 km transect, to five and seven clearly illustrate this. However, the current situation is slightly better than about 12 months ago when 53 woylies were removed temporarily to Monarto. Thirty have since been returned. It should also be taken into consideration that the peak numbers from two years ago were abnormally high and obviously totally unsustainable.

Unfortunately monitoring by sample trapping was not underway at the time of the population peak indicated by spotlight counts in mid 2005, having started later that year. Trapping showed a drop from 17 from 40 traps in November 2005, to three from 40 at the time the dramatic reduction in spotlight counts numbers took place in mid 2006, then a rise to ten from 40 traps this month (September 2007). The trap results are not as graphic as the spotlight counts in showing the drop in woylie numbers, but woylie trappability is high and traps are effective for the whole night. The distance woylies are attracted to the oat trail used in the spotlight counts are not known. Considering these factors, it is still thought that the population decline is in excess of 90%.

4.7.3. Possible cause of the decline

Woylie numbers began to fall in the lower half of the peninsula about mid 2005, initially due to exhaustion of principle food resources resulting from over population. When the numbers declined past a critical point where woylie reproduction was not exceeding cat predation, the cat predation would have created an accelerated decline. This combination of reduced food availability and a higher percentage of animals being lost to predation were probably gradually expanding up the peninsula. But, the final blow for the population was a series of six frosts in a week in June 2005. Frosts are infrequent in the area, with only one recorded over three previous winters. A combination of limited food resources, cat predation and this severe weather event is currently the most logical explanation available for the extreme population decline.

It was not possible to test the theory that severe frosts may have caused the sudden deaths of a large number of woylies for several reasons. Their bodies would not be obvious as they would have been in their carefully concealed nests beneath low shrubs. The only way to locate them would have been with dogs, which could have detected the decomposing bodies by scent. Unfortunately, due to the large number of 1080 baits throughout the area, a well-trained, muzzled and reliable dog would have been required and the time to carry out this task was limited. No such dog was readily available and the frosts were several weeks before the drop in numbers was known.

4.7.4. When the woylies were sent to Monarto

The spotlight count which first alerted to the decline in woylie numbers was carried out on 4/7/06. The decision to move animals to Monarto was made on 6/7/06. Between 6/7/06 and 11/7/06 at total of 53 (32 males, 21 females) were transported to Monarto for safekeeping and health checks. Twenty of these (12 males, 8 females) were returned on 20/12/06 and released in the general area of their initial capture. Another ten (eight males, two females) were returned and released in the same area on 4/6/07. Of these, four were pouch-young born and/or raised at Monarto. Monitoring of ten of the first group of 20 by radio tracking showed all ten survived until transmitters were removed in mid to late April 2007. All but one of the other ten were trapped at least once post release.

CHAPTER 5 DISEASE

5.1. Rationale to the approach to disease investigation

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Abstract

The Woylie Disease Reference Council (WDRC) was established to provide a rapid response to the need to identify the possible role(s) of disease in recent woylie declines. The WDRC was designed to efficiently and effectively co-ordinate the complexity of a multidisciplinary and highly collaborative disease investigation including haematology, bacteriology, parisitology virology, toxicology, pathology, nutrition and clinical investigations, epidemiology, and genetics. The broad objectives of the disease investigations associated with the Woylie Conservation Research Project (WCRP) are outlined. The rationale for the WCRP approach, its charter and membership are provided. The general strategies for the disease investigations are listed. In addition to successfully addressing the needs of the WCRP, this approach provides a valuable model for future investigations on disease-related wildlife issues in Western Australia.

5.1.1. Introduction

Australia has the worst modern mammal extinction rate in the world and currently 20% of the remaining mammal species are threatened with extinction. Disease is known to be a primary threat associated with at least 11% of declining vertebrate species (e.g. Yiming and Wilcove, 2005). The Tasmanian devil facial tumour disease, chytridimycosis in many frog species, *Chlamydia* in koalas, *Papilloma* virus in western barred bandicoots, and *Treponema* (human syphilis) in Gilbert's potoroo are just some Australian examples of these cases (Warren *et al.*, 2005). Wildlife diseases as a source of zoonosis are also significant human health issues, including avian influenza, SARS, lyssavirus in some bats, *Rickettsia*, Leptospirosis, hydatid disease, *Salmonella*, Psittacosis, Arboviruses (e.g. Ross River, Murray Valley encephalitis), toxoplasmosis, and mycobacteriosis.

Little is known about the present disease status of Western Australia's native fauna. What information is available is sparse, fragmentary and incomplete, and has largely been opportunistically derived from carcasses. The extent to which infectious diseases represent a threat to wildlife populations in WA will depend upon a complex interplay between characteristics of both the host and the infectious agent which include viruses, bacteria, protozoa and macroparasites, such as helminths and arthropods. The most important host characteristics are population size, density and migration rate.

Infectious diseases may, at least partially, be responsible for the most recent declines in Western Australian small mammal fauna. There is some evidence to support this view: the declines are widespread, but species-selective; they have occurred in the face of continuing fox control through the *Western Shield* program; there are no obvious correlations between the declines and abiotic factors; and preliminary surveys have identified high prevalences of parasitic infection in some populations of some species.

The disease agents potentially responsible for marsupial population declines (either in isolation or in concert with other factors) are extensive and can be grouped as;

1. viral
2. bacterial
3. haemoparasites
4. endoparasitic
5. ectoparasitic
6. toxic
7. nutritional

Other disease-related disciplines include;

8. haematology
9. pathology
10. clinical medicine
11. genetics
12. epidemiology

5.1.2. WCRP objectives for disease investigation

1. Identify parasites and other micro-organisms and evaluate their potential role in woylie declines.
2. Use expertise to prioritise which known and unknown diseases may be associated with woylie declines.
3. Examine indirect evidence (e.g. demographic changes) that may help to determine whether a disease in general or a specific disease may be responsible for recent woylie declines.
4. Assess the prevalence and potential for specific high-risk disease agents to be a causal factor in the decline of woylies.

5.1.3. The Woylie Disease Reference Council (WDRC)

The woylie decline represented a unique opportunity to address a wildlife decline at the outset, using the full breadth of multidisciplinary expertise available in Western Australia. Thus clinicians, ecologists, geneticists, epidemiologists, infectious disease specialists, nutritionists and pathologists were brought together to work on the problem collectively and cooperatively, in terms of planning, execution and interpretation. The Woylie Disease Reference Council (WDRC) has been established with expertise from Murdoch University and Perth Zoo in association with representation from DEC. Such a group response is quite different to bringing in individual expertise only when required for specific data collection, sample analysis, etc.

The rationale for a disease reference council model over other options includes;

- Considerations of the size and complexity of the issues and the short timeframe in which to respond and assess the possible roles of disease while declines were occurring.
- A strength of the council model is the direct involvement of the specialist expertise within each of the disease subdisciplines, which collectively provide an indepth and comprehensive treatment of the major components within wildlife health and disease.

The charter for the WDRC is;

1. Be the principle forum for addressing woylie disease issues.
2. Assist in the collation of existing information on woylie/wildlife diseases in Western Australia
3. Provide expert advice and direction on research priorities into putative disease agents of woylie/wildlife declines
4. Facilitate and develop collaborative endeavours into woylie/wildlife disease research between DEC WCRP staff and disease experts and students.

5.1.3.1. Key WDRC Roles

Woylie Conservation Research Project Co-ordinator	Dr Adrian Wayne
Woylie Disease Reference Council Chair	Prof Andrew Thompson
WDRC Clinical Co-ordinator	Dr Paul Eden
WDRC Pathology Co-ordinator	Dr Graeme Knowles
WDRC Secretary	Dr Halina Burmej

5.1.3.2. WDRC Membership

Prof Andrew Thompson - Parasitology / Principal Murdoch liaison / WDRC chair
A/Prof Stan Fenwick – Microbiology and Public Health
*A/Prof Phil Clark – Clinical Pathology (Haematology)
Dr Phil Nicholls –Pathology
*Dr Graeme Knowles – Pathology / WDRC Pathology Co-ordinator
A/Prof Alan Lymbery – Parasitology and Ecology
Dr Andy Smith – Parasitology and Ecology (ectoparasites)
Dr Peter Adams – Parasitology and Microbiology
Dr Trevor Ellis – Virologist
A/Prof Ian Robertson – Epidemiologist
Cree Monaghan – Wildlife Clinician and Nutritionalist
Paul Eden – Wildlife Clinician / WDRC Clinical Co-ordinator
Carlo Pacioni – PhD Candidate (Molecular genetics)
Nevi Parameswaran – PhD Candidate (Toxoplasmosis in wildlife)
Unaiza Parkar – PhD Candidate (Endoparasites in wildlife)
Yazid Abdad – PhD Candidate (Ectoparasites and *Rickettsia* in wildlife)
*Lisa Hulme-Moir – PhD Candidate (Clinical pathology)
Halina Burmej – PhD Candidate (Ectoparasites in wildlife)
Keith Morris – Fauna Conservation Program Leader (DEC)
Dr Adrian Wayne –WCRP Co-ordinator and Chief Investigator (DEC)
Marnie Swinburn – Warren Region Fauna Conservation Officer (DEC)
<u>Recent membership appointments;</u>
Susana Averis – Trypanosome genetic characterisation
Andrea Reiss - Wildlife Clinician (Biochemistry)
Peter Irwin – Haemaparasites (Piroplasms)

* = recent retirements.

5.1.4. Strategies for addressing disease investigation

- Collation of existing information on woylie/wildlife diseases in WA to develop baseline information for clinically healthy animals in apparently stable populations.
- Development of a comprehensive list of wildlife diseases and an expertise-based risk assessment of their potential as a causal agent in the decline of woylies in southwestern Australia.
- Population screening to identify what diseases may be present and their prevalence in the Upper Warren, Karakamia, and other potentially valuable comparative sites.

- Disease/health profiles of individuals radio-collared and monitored as part of the survivorship and mortality research for direct comparison if/when the individuals are recovered dead.
- Disease/health monitoring and epidemiology as part of the associated trapping programs (i.e. Upper Warren Fauna Monitoring and PCS - density and demographics assessments; trapping programs at other woylie populations in WA and SA).
- Specific disease investigations as evidence become available.
- Diagnosis of sick, moribund and dead animals as they are encountered during other woylie conservation research activities and otherwise encountered opportunistically.
- Collect reference blood serum and other samples that will be stored indefinitely or until new information presents sufficient cause to undertake further analysis and/or specific tests.
- Post-mortem examination to identify factors contributing to the mortality of woylie study subjects and bodies that may opportunistically be recovered. Such post-mortem examinations provide immediate results from gross and light microscopy, but also build an archive of other tissues that can later be used for testing when a candidate agent is proposed.
- Collection and analysis of tissue samples to examine genetic attributes associated with declining and remnant woylie populations.

5.1.5. Discussion

The multidisciplinary and highly collaborative investigations into the potential role of disease in recent woylie declines are effectively and efficiently co-ordinated through the WDRC. Collectively these efforts also serve to substantially improve the knowledge base of diseases, parasites and other infectious agents associated with woylies and sympatric species within southwestern Australia and South Australia.

Identifying what parasites and diseases are present within woylie populations across space and time is the first step to understanding their potential association with woylie declines. Understanding their capacity to facilitate population declines is the second step. In addition to highly pathogenic agents, it is as important to consider infectious agents that may affect behaviour, condition and fecundity in a manner that can increase vulnerability to predation and/or other factors that result in increased mortality or reduced recruitment, given that these too are capable of driving declines such as those observed in woylies.

The very large amount of disease data and information generated within a very short time frame is direct evidence of the effectiveness of the WDRC model and approach used in the WCRP. Subsequent sections of this report describe much of the progress made to date in relation to the many parallel disease investigations underway.

The success of WDRC serves as a model for future investigations on wildlife issues in Western Australia, particularly when disease is believed to be a contributing factor. The WDRC has already played a significant role in the establishment and operations of the collaborative (Murdoch University and DEC) ARC research project “The nature, diversity and potential impact of infectious agents in Western Australian threatened mammals” – funded by an ARC Linkage grant. The activities of the WDRC have, and will continue, to also offer excellent experience and training in wildlife conservation for research students.

5.1.6. References

Yiming, L. and Wilcove, D.S., 2005. Threats to vertebrate species in China and the United States, *BioScience* **55(2)** 147-153.

Warren, K. *et al.* (2005) Ocular *Chlamydiales* infections of western barred bandicoots (*Perameles bougainville*) in Western Australia. *J. Zoo Wildl. Med*. **36**: 100-102.

5.2. Field health and disease sampling

Adrian Wayne, Marnie Swinburn, Colin Ward, Julia Wayne, Brian Whittred, Marika Maxwell, Chris Vellios

Department of Environment and Conservation, Manjimup'

Abstract

Routine health checks and sampling for disease analyses were conducted on all woylies on Upper Warren fauna monitoring transects and Population Comparison Study sites. The principal benefit of the health checks is the increased likelihood of the detection of individuals displaying clinical symptoms that may provide clues or direct evidence of factors associated with recent woylie declines. Preliminary exploration indicates substantial differences in the prevalence of eye and skin conditions. Whether these differences are related to observer differences or associated with woylie declines remains to be investigated.

The routine sampling in the field of blood, faeces and ectoparasites from woylies for disease investigations is summarized. The history of sampling of radio-collared individuals associated with the 'Population Comparison Study' of woylie survivorship and mortality is also briefly summarized. Samples from radio-collared cohorts include biometrics, health checks and disease samples.

5.2.1. Introduction

Routine health checks and sampling for disease analyses were conducted on all woylies and some sympatric species trapped on Upper Warren fauna monitoring transects and Population Comparison Study sites. The field health checks constitute the best opportunity for detecting clinical symptoms of disease and assessing the general condition of animals more rigorously, and in more detail than has customarily been done during general fauna monitoring and research. This health check process also identifies individuals that may require additional disease sampling and/or referral to wildlife clinicians for further examination.

Field samples from fauna (blood, ectoparasites, faeces, tissue) for disease, food resource and genetic investigations by collaborating specialists were collected by field staff under the general direction of the Woylie Disease Reference Council (WDRC).

5.2.2. Methods

Details of the protocols associated with the field health checks and the sampling of blood, ectoparasites, faeces, and tissue are provided in the WCRP Operations Handbook (Volume 3). Procedural familiarization and skill development of nominated animal handlers performing the field health checks and sampling were primarily delivered via pre-trapping inductions, on-the-job training and a training day at Murdoch University. Training was provided by Adrian Wayne, visiting veterinarians and vet nurses assisting in the field and at a training day presented by technical and specialist expertise associated with the WDRC at Murdoch and Perth Zoo. The assessment of field examination and sampling competencies were conducted by Adrian Wayne. Improvements were made over time and where necessary in response to feedback from the technicians processing the samples, ongoing procedural reviews, trapping debriefings and collective learning exercises. Standardisation and consistency between operators was promoted by having a single assessor and procedural reviews (individuals and groups).

5.2.3. Results

5.2.3.1. Field health checks

A total of 173 and 740 health checks of woylies were recorded at Karakamia and Upper Warren respectively (Table 5.2.1). Preliminary exploration of the data indicates that skin conditions were the most commonly recorded observations (Figure 5.2.1.). Dermatitis (flaky and/or scaly skin), particularly on the rump, back and dorsal tail base were most prevalent. Scratches, dry lesions and scabbing were also frequently reported, especially on the back and rump (Figure 5.2.1.d&e). Most notable is the substantially higher reporting incidence at Balban and to a lesser extent, Winnejup of fur loss and/or scabbing around the eye (Figure 5.2.1.b), dermatitis and scratches, dry lesions and scabs. Approximately 40% of all health check records from these two sites were collected from one individual who worked almost exclusively on these two sites. This dataset remains to be fully examined and analysed.

Figure 5.2.1. Examples of some of the conditions reported in the woylie field health checks.

a) Ectoparasites (ticks) in the ear, b) hair loss and scabbing around the eye, c) lesions and scarring around mouth, d&e) hair loss and scabbing on the back and rump, f) hair loss and scabbing on the tail

Table 5.2.1. Preliminary summary of selected incidences of woylie health records, body condition and coat condition (%).

Coat Condition and Body Condition indices are from woylies assessed between 11th July 2006 and 13th September 2007. Health conditions are from woylie field health checks performed from 11th July 2006. Dermatitis is the combined description of flaky, scaly or scabby skin.

	Karakamia (n=173)	Keninup (n=376)	Warrup (n=168)	Balban (n=98)	Winnejup (n=23)	Boyicup (n=17)	Corbal (n=33)	Chariup (n=11)	Moopinup (n=9)	Yendicup (n=3)	Tutanning (n=2)	Total (%) (n=913)
Eye Conditions												
fur loss and/or scabbing around eye	0.6	5.6	3.6	34.7	30.4			9.1	11.1			**7.8**
cloudy/cataracts	6.4	1.3	0.6	3.1	8.7							**2.4**
pus/discharge	0.6			1.0		5.9						**0.3**
Mouth												
scabbing around mouth		0.3		4.1	4.3							**0.7**
lower lip coming away				1.0			3.0					**0.2**
Dermatitis												
location not specified	4.6	2.4	3.6	44.9	8.7	5.9			1.0			**7.8**
back/rump		2.9	5.4			11.8	3.0					**2.5**
tail	1.7	10.1	7.7	34.7	39.1		6.1	9.1	11.1			**11.1**
Fur loss												
location not specified		1.3	1.2	3.1	4.3							**1.2**
back/rump		6.9	3.6	6.1	8.7							**4.4**
tail	0.6	5.9	2.4	9.2	17.4		12.1	9.1				**4.9**
Scratches, dry lesions, scabs												
location not specified	0.6	0.5	1.2	4.1	4.3		3.0					**1.2**
back/rump		2.1	6.0	13.3	17.4		3.0					**3.9**
tail	2.9	0.8	0.6	3.1								**1.3**
Body Condition Indices	n=175	n=428	n=210	n=100	n=30	n=19	n=34	n=10	n=9		n=7	**Total (%) (n=1022)**
1-2	8.6			1.0								**1.8**
3	58.9	21.0	8.1	28.0	16.7	5.3	2.9	10.0	11.1			**27.1**
4-5	32.6	79.0	91.9	71.0	83.3	94.7	97.1	90.0	88.9		100.0	**83.1**
Coat Condition Indices	n=175	n=427	n=209	n=99	n=30	n=18	n=34	n=10	n=9		n=7	**Total (%) (n=1018)**
1-2	1.1	1.2	1.0	2.0			2.9					**1.3**
3	23.4	11.0	9.6	18.2	23.3	38.9	2.9	10.0	11.1		14.3	**15.8**
4-5	75.4	87.8	41.6	79.8	76.7	61.1	94.1	90.0	88.9		85.7	**94.4**

5.2.3.2. Disease Sampling

Table 5.2.2 summarises the number of disease samples collected in the field as part of the WCRP, with Table 5.2.3 summarising those samples collected from the Upper Warren. Sampling within the Upper Warren occurred principally at the five PCS sites, with repeat sampling events over time to provide information on any possible spatial and/or longitudinal changes in disease prevalence (Table 5.2.4). In these tables, haematology sample numbers are based on results received from ClinPath prior to January 2008. Additional haematology results have since been received but have not yet been summarised. As of 30th January 2008, 633 haematology samples had been received by ClinPath, 511 of which were analysed. 122 samples were discarded due to clotting or insufficient quantities for analysis.

'Reference' serum from all trapping programs are in frozen storage at Murdoch University (care of Nevi Parameswaran) for future analyses as they may be needed and in response to any new evidence that might merit further investigation (e.g. screening for selected virus types). The numbers of reference sera collected are presented in Table 5.2.5. Tissue samples for genetic research and faeces for dietary analysis are addressed in the report elsewhere (Chapter 6 Conservation genetics and Section 4.5 Resources).

Table 5.2.2. The recorded number of woylie disease samples collected from trapping programs between March 2006 and November 2007.

	Haematology	Toxoplasma	Ectoparasites	Endoparasites	Salmonella
Upper Warren	429 (286)	474 (404)	767 (11*)	562 (68)	140 (137)
Karakamia	37 (37)	91 (81)	262	99 (62)	27
Batalling	26 (23)	30 (26)	26	21	0
Tutanning	5 (4)	8 (8)	6	4	0
Dryandra	12 (4)	13 (12)	12	6	0
South Australia	129 (89)	129 (86)	81	89 (42)	0
TOTAL	**629 (443)^**	**745 (617)**	**1154 (11*)**	**781 (172)**	**167 (137)**

Parentheses indicate number of samples reported to have been analysed to date.
^ Number of samples reported to have been analysed prior to January 2008
* indicate samples analysed for *Rickettsia*

Table 5.2.3. Number of woylie samples collected from the Upper Warren between March 2006 and November 2007.

	Haematology	Toxoplasma	Ectoparasites	Endoparasites	Salmonella
Balban	84	87	127	117	44
Boyicup2	12	13	17	13	4
Chariup	11	12	16	14	5
Corbal	32	33	50	55	26
Keninup2	152	173	355	212	18
Moopinup	6	6	9	7	0
Warrup2	99	116	153	114	31
Winnejup	23	24	27	22	7
Yendicup2	10	10	13	8	5
TOTAL	**429^**	**474**	**767**	**562**	**140**

Bold denotes PCS sites
^Haematology data available prior to January 2008

Table 5.2.4. Sample collection matrix for Upper Warren and Population Comparison Study sites.

EDTA, Plain and Smear= blood, Diet = Faeces for dietary analysis, Endo=Faeces for endoparasite analysis, Ecto= Ectoparasites, DNA= tissue (ear biopsy), Sal= Salmonella.

Sites		Mar-06	Apr-06	Jul-06	Aug-06	Sep-06	Oct-06	Nov-06	Dec-06	Jan-07	Feb-07	Mar-07	Apr-07	May-07	Jun-07	Jul-07	Nov-07		Total
Balban	EDTA	40		8	1	0	6	7	2		1	14	0	0			5	EDTA	**84**
	Plain	42		8	1	0	6	7	2		0	15	0	0			5	Plain	**86**
	Diet	50		14	2	9	18	10	7		6	30	5	3			5	Diet	**159**
	Endo	48		19	2	0	6	7	2		1	30	1	0			1	Endo	**117**
	Ecto	43		12	0	6	13	6	7		5	25	4	3			3	Ecto	**127**
	DNA	0		8	1	1	8	10	0		3	17	0	0			0	DNA	**48**
	Smear	0		9	1	0	6	5	2		1	16	0	0			5	Smear	**45**
	Sal	43		0	0	0	0	0	0		1	0	0	0			0	Sal	**44**
Boyicup2	EDTA	4		1	0	0	0	4	0		0	3	0					EDTA	**12**
	Plain	4		1	1	0	0	4	0		0	3	0					Plain	**13**
	Diet	5		1	0	2	2	4	2		2	3	1					Diet	**22**
	Endo	5		1	0	0	0	4	0		0	3	0					Endo	**13**
	Ecto	5		1	1	1	1	3	0		1	3	1					Ecto	**17**
	DNA	0		1	1	1	0	2	0		0	2	0					DNA	**7**
	Smear	0		0	1	0	0	4	0		0	3	0					Smear	**8**
	Sal	4		0	0	0	0	0	0		0	0	0					Sal	**4**
Chariup	EDTA	4						0				7						EDTA	**11**
	Plain	6						0				6						Plain	**12**
	Diet	6						4				6						Diet	**16**
	Endo	5						3				6						Endo	**14**
	Ecto	6						3				7						Ecto	**16**
	DNA	0						4				5						DNA	**9**
	Smear	0						0				5						Smear	**5**
	Sal	5						0				0						Sal	**5**
Corbal	EDTA	25						0					7					EDTA	**32**
	Plain	27						0					8					Plain	**35**
	Diet	27						20					13					Diet	**60**
	Endo	25						19					11					Endo	**55**
	Ecto	21						17					12					Ecto	**50**
	DNA	0						20					8					DNA	**28**
	Smear	0						0					8					Smear	**8**
	Sal	26						0					0					Sal	**26**
Keninup2	EDTA	20		8	4	0	17	13	9		0	29	0	8	1		43	EDTA	**152**
	Plain	35		11	4	0	17	16	10		0	30	0	9	1		48	Plain	**181**
	Diet	36		16	10	28	47	30	28		37	81	33	22	2		37	Diet	**407**
	Endo	36		17	10	0	19	23	8		0	81	0	0	0		18	Endo	**212**
	Ecto	49		15	4	19	30	29	25		31	75	22	23	2		31	Ecto	**355**
	DNA	0		11	3	10	28	69	12		5	50	5	3	0		28	DNA	**224**
	Smear	0		6	4	0	16	15	7		0	29	8	0	1		25	Smear	**111**
	Sal	18		0	0	0	0	0	0		0	0	0	0	0		0	Sal	**18**
Moopinup	EDTA							0				6						EDTA	**6**
	Plain							0				6						Plain	**6**
	Diet							2				6						Diet	**8**
	Endo							1				6						Endo	**7**
	Ecto							3				6						Ecto	**9**
	DNA							3				5						DNA	**8**
	Smear							0				6						Smear	**6**
	Sal							0				0						Sal	**0**
Warrup2	EDTA	20		9	0	0	0	29	0		0		13	0			28	EDTA	**99**
	Plain	23		11	0	1	0	28	0		0		20	0			37	Plain	**120**
	Diet	30		10	1	5	3	37	5		20		63	10			32	Diet	**216**
	Endo	31		10	1	0	0	34	0		0		37	0			1	Endo	**114**
	Ecto	26		11	1	1	0	34	1		18		54	7			0	Ecto	**153**
	DNA	0		11	0	4	1	29	2		6		29	1			11	DNA	**94**
	Smear	0		10	0	0	0	30	0		0		17	0			28	Smear	**85**
	Sal	31		0	0	0	0	0	0		0		0	0			0	Sal	**31**
Winnejup	EDTA	8		2	2	3	0	4				4	0	0				EDTA	**23**
	Plain	2		2	2	3	0	4				4	0	0				Plain	**17**
	Diet	10		2	3	2	1	4				0	8	2				Diet	**32**
	Endo	10		1	2	0	0	4				0	5	0				Endo	**22**
	Ecto	6		2	1	3	1	4				0	9	1				Ecto	**27**
	DNA	0		2	2	3	0	2				0	3	0				DNA	**12**
	Smear	0		1	0	0	0	3				0	5	0				Smear	**9**
	Sal	7		0	0	0	0	0				0	0	0				Sal	**7**
Yendicup2	EDTA		8				0					2						EDTA	**10**
	Plain		8				0					2						Plain	**10**
	Diet		5				3					3						Diet	**11**
	Endo		5				0					3						Endo	**8**
	Ecto		7				4					2						Ecto	**13**
	DNA		0				4					3						DNA	**7**
	Smear		0				0					2						Smear	**2**
	Sal		5				0					0						Sal	**5**
Karakamia	EDTA			0		0	18	0		0		0		0		3	7	EDTA	**28**
	Plain			86		0	0	0		0		0		0		3	7	Plain	**96**
	Diet			0		15	0	18		40		27		36		28	32	Diet	**196**
	Endo			62		0	0	0		0		27		0		0	10	Endo	**99**
	Ecto			41		33	0	15		32		28		34		39	40	Ecto	**262**
	DNA			88		0	0	0		40		13		6		4	3	DNA	**154**
	Smear			0		0	0	0		0		0		0		3	7	Smear	**10**
	Sal			0		0	0	0		0		27		0		0	0	Sal	**27**
TOTAL		**874**	**38**	**529**	**66**	**150**	**275**	**650**	**131**	**112**	**138**	**772**	**410**	**168**	**7**	**80**	**497**		**4897**

Bold indicates PCS sites

Table 5.2.5. Number of reference sera collected between March 2006 and November 2007 from each trapping program

Site	Total # Reference sera
Upper Warren	444
Karakamia Sanctuary	74
Batalling	30
Dryandra	8
Tutanning	13
South Australia	87
TOTAL	**656**

5.2.3.3. Radio-collared woylie cohorts

The radio-collared cohorts at the five Upper Warren PCS sites are a subset of the woylies that have been examined and sampled in these areas. The incidence of data collection (biometrics and field health checks) and disease sample collection (blood, faeces and ectoparasites) of radio-collared individuals is provided in Tables 5.2.6 and 5.2.7 respectively.

Table 5.2.6. Summary of the incidents of biometric ('M') and field health check ('H') data recorded for woylie individuals radio-collared and monitored as part of the survivorship and mortality component of the Population Comparison Study.

Site	Animal Record#	Nov-04	Dec-04	Jan-05	Feb-05	Mar-05	Apr-05	May-05	Jun-05	Jul-05	Aug-05	Sep-05	Oct-05	Nov-05	Dec-05	Jan-06	Feb-06	Mar-06	Apr-06	May-06	Jun-06	Jul-06	Aug-06	Sep-06	Oct-06	Nov-06	Dec-06	Jan-07	Feb-07	Mar-07	Apr-07	May-07	Jun-07	Fate
Balban	09-0-000-801	M												M				M				MH	MH	MH	MH		MH		MH					Removed 12/02/2007
Balban	09-0-000-862													M				M				MH		MH										Died 29/09/2006
Balban	09-0-000-893													M				M				MH		MH	M	MH								Died 4/12/2006
Balban	09-0-000-894													M				M				MH		MH										Died 5/10/2006
Balban	50-0-003-181	M												M				M				MH		MH	MH		MH		MH					Died 09/04/07
Balban	50-0-003-354																	M				MH		MH	M									Removed 5/09/2006
Balban	50-0-003-733																								MH		MH							Died 30/01/2007
Balban	50-0-003-884																					MH		MH										Died 13/09/2006
Balban	50-0-003-887																					M		MH	MH									Died 8/11/2006
Balban	50-0-003-894																					MH												Died 12/08/2006
Balban	50-0-003-911																						MH	MH	MH		MH		MH		MH	MH		Removed 12/12/2006
Boyicup	50-0-002-272					M								M									MH											Died 24/10/2006
Boyicup	50-0-003-741																					MH		MH	M	MH	MH		MH	MH	MH			Removed 6/09/2006
Keninup	50-0-002-539													M				M				MH	M	M	M	MH	MH		MH	MH	MH	MH		Removed 12/02/2007
Keninup	50-0-002-574													M								MH		MH	MH				M					Died 26/02/2007
Keninup	50-0-002-598													M								MH		MH	MH	MH	MH		MH	MH	MH	MH		Removed 29/05/2007
Keninup	50-0-003-221																	M				MH		MH	MH	MH	MH		M	MH	MH			Died 2/06/2007
Keninup	50-0-003-222																	M				MH		MH	M		MH		MH		MH	Off air		Last trapped 13/04/07
Keninup	50-0-003-789																					MH		MH							MH	MH		Removed 5/09/2006
Keninup	50-0-003-790																					MH		MH	MH	M	MH		MH	MH	MH	MH		Removed 31/05/2007
Keninup	50-0-003-792																					MH		MH	MH									Died 15/11/06
Keninup	50-0-003-793																					MH		MH	H		MH		MH		MH			Removed 14/01/2007
Keninup	50-0-003-797																					MH												Died 29/08/2006
Keninup	50-0-003-801																					MH												Died 19/08/2006
Keninup	50-0-003-807																					MH	MH	MH	MH		MH		MH		MH	MH		Removed 29/05/2007
Keninup	50-0-003-815																						M	M	M		MH		MH		MH	MH		Removed 11/04/2007
Keninup	50-0-003-816																						MH	M	MH		MH		MH		MH	MH		Removed 30/05/2007
Keninup	50-0-003-819																						MH		MH		MH		H	M	MH			Removed 8/06/2007
Keninup	50-0-003-826																							MH	M		MH		MH		MH	MH		Removed 30/05/2007
Keninup	50-0-003-829																							M	M		MH							Removed 12/06/2007
Keninup	50-0-003-833																								MH		MH		M		MH	MH		Removed 29/05/2007
Keninup	50-0-003-842																								MH		MH		M			MH		Removed 31/05/07
Keninup	50-0-003-851																								M		M		M		M			Removed 12/02/2007
Keninup	50-0-003-859																										MH		M		MH	MH		Removed 12/02/2007
Keninup	50-0-003-867																										MH		MH		MH	MH		Removed 29/05/2007
Keninup	50-0-003-873																										MH		MH		MH	MH		Removed 29/05/2007
Keninup	50-0-003-929																						MH											Died 11/08/2006
Keninup	50-0-006-580																							M										Removed 5/09/2006
Warrup	50-0-002-307						M								M			M								MH				MH	MH	H		Removed 5/09/2006
Warrup	50-0-003-015																	M				MH		M	M		MH		MH					Died 30/03/2007
Warrup	50-0-003-072																	M				MH		M										Died 21/10/2006
Warrup	50-0-003-134													M				M				MH	M	M	M	MH	MH		MH		MH	MH		Removed 25/10/2006
Warrup	50-0-003-163													M								MH	M	M	M		MH		MH		MH	MH		Removed 6/09/2006
Warrup	50-0-003-188													M				M				MH		M	M	MH	MH		MH		MH	MH		Removed 12/02/2007
Warrup	50-0-003-193													M				M				MH			M	MH								Removed 25/10/2006
Warrup	50-0-003-834																					MH	M	M	M		MH		MH		MH	M		Removed 30/05/2007
Warrup	50-0-003-847																					MH		M	M		MH		MH		MH	MH		Removed 29/05/2007
Warrup	50-0-003-852																					MH		M	M	MH			MH		MH	MH		Removed 5/09/2006
Warrup	50-0-003-853																					MH		MH	M		M							Died 26/02/2007
Winnejup	00-0-003-715													M				M						MH	M	MH	MH		MH		MH			Died 21/05/2007
Winnejup	00-0-003-736																	M				MH	M	M		MH					MH	MH		Removed 29/05/2007
Winnejup	50-0-003-753																					MH												Died 22/07/2006
Winnejup	50-0-003-760																						MH	MH										Died 21/10/2006
Winnejup	50-0-003-766																						MH	M	M									Removed 6/06/2007
Winnejup	50-0-003-780																							H							MH			Removed 29/05/2007

Note: Shaded cells indicate the period when individuals were collared with mortality-sensitive radio-transmitters.

Table 5.2.7. Summary of the incidents of sampling for disease analyses (B=blood, F=faeces, E=ectoparasites) recorded for woylie individuals radio-collared and monitored as part of the survivorship and mortality component of the Population Comparison Study.

Site	Animal Record#	Mar-06	Apr-06	May-06	Jun-06	Jul-06	Aug-06	Sep-06	Oct-06	Nov-06	Dec-06	Jan-07	Feb-07	Mar-07	Apr-07	May-07	Jun-07	Fate
Balban	09-0-000-801	BFE				BFE		F	BFE		BFE							Removed 12/02/2007
Balban	09-0-000-862	BFE				BFE		FE										Died 29/09/2006
Balban	09-0-000-893					BFE		F		F								Died 4/12/2006
Balban	09-0-000-894	FE				BFE		F										Died 5/10/2006
Balban	50-0-003-181	FE				BFE	F	FE	BFE		FE		FE					Died 09/04/07
Balban	50-0-003-354	BFE				FE		FE	F									Removed 5/09/2006
Balban	50-0-003-733								FE		BFE							Died 30/01/2007
Balban	50-0-003-884					BFE												Died 13/09/2006
Balban	50-0-003-887					BFE		E	FE									Died 8/11/2006
Balban	50-0-003-894					BFE												Died 12/08/2006
Balban	50-0-003-911						BF	E	F		FE		FE		FE	FE		Removed 12/12/2006
Boyicup	50-0-002-272						BE											Died 24/10/2006
Boyicup	50-0-003-741					BFE		F	FE	BF	FE		F	BFE	F			Removed 6/09/2006
Keninup	50-0-002-539	FE					F	FE	FE	BF	FE		F	BFE	F	FE		Removed 12/02/2007
Keninup	50-0-002-574					BFE		FE	BFE				F					Died 26/02/2007
Keninup	50-0-002-598					BFE		F	FE	BF	FE		FE	BFE	F	BFE		Removed 29/05/2007
Keninup	50-0-003-221	BE				BFE		FE	F	BFE	FE		F	BFE	FE			Died 2/06/2007
Keninup	50-0-003-222					BFE		F	FE		BFE		F			Off		Last trapped 13/04/07
Keninup	50-0-003-789					BFE	F	FE										Removed 5/09/2006
Keninup	50-0-003-790					BFE		F	F	FE	BF		FE	BFE		BFE	B	Removed 31/05/2007
Keninup	50-0-003-792					BFE			F									Died 15/11/06
Keninup	50-0-003-793					BFE		FE	FE		E		FE		FE			Removed 14/01/2007
Keninup	50-0-003-797					BFE												Died 29/08/2006
Keninup	50-0-003-801					BFE												Died 19/08/2006
Keninup	50-0-003-807					BFE	BFE	F	FE	FE			FE		FE	BFE		Removed 29/05/2007
Keninup	50-0-003-815						F	FE	F		BFE		FE		F	FE		Removed 11/04/2007
Keninup	50-0-003-816						BFE	FE	F		FE		FE		F	BFE		Removed 30/05/2007
Keninup	50-0-003-819						BFE		FE		F		F		F			Removed 8/06/2007
Keninup	50-0-003-826							FE	F		BFE		FE		F	BFE		Removed 30/05/2007
Keninup	50-0-003-829							FE	F		BF							Removed 12/06/2007
Keninup	50-0-003-833										BFE		FE		FE	BFE		Removed 29/05/2007
Keninup	50-0-003-842								F		BF							Removed 31/05/2007
Keninup	50-0-003-851								FE		BFE		FE		FE			Removed 12/02/2007
Keninup	50-0-003-859										BFE		FE		FE	FE		Removed 12/02/2007
Keninup	50-0-003-867										FE		FE		FE	BFE		Removed 29/05/2007
Keninup	50-0-003-873										BFE		FE		F	BF		Removed 29/05/2007
Keninup	50-0-003-929						BE											Died 11/08/2006
Keninup	50-0-006-580							FE										Removed 5/09/2006
Warrup	50-0-002-307					BFE				BFE			FE		BFE	FE		Removed 5/09/2006
Warrup	50-0-003-015	BF				BFE					F		FE					Died 30/03/2007
Warrup	50-0-003-072					BE												Died 21/10/2006
Warrup	50-0-003-134	BFE				BFE				BFE	F		FE		FE	FE		Removed 25/10/2006
Warrup	50-0-003-163					BFE		F					FE		F	FE		Removed 6/09/2006
Warrup	50-0-003-188					BE				BF			FE		BFE	E		Removed 12/02/2007
Warrup	50-0-003-193					BE				BFE								Removed 25/10/2006
Warrup	50-0-003-834					BFE												Removed 30/05/2007
Warrup	50-0-003-847					BFE							FE		FE	FE		Removed 29/05/2007
Warrup	50-0-003-852					BFE			F	F			FE		BFE	F		Removed 5/09/2006
Warrup	50-0-003-853					F		BFE										Died 26/02/2007
Winnejup	00-0-003-715	BFE						BFE		BFE					BFE			Died 21/05/2007
Winnejup	00-0-003-736	BF				BFE				BFE					BFE	FE		Removed 29/05/2007
Winnejup	50-0-003-753					BFE												Died 22/07/2006
Winnejup	50-0-003-760						BF	BFE										Died 21/10/2006
Winnejup	50-0-003-766						BFE		FE									Removed 6/06/2007
Winnejup	50-0-003-780							BE							FE			Removed 29/05/2007

Note: Shaded cells indicate the period when individuals were collared with mortality-sensitive radio-transmitters.

5.2.4. Discussion

5.2.4.1. Health check records

The data generated by the field health checks remain to be fully examined. Likely useful information to be generated from these data and the associated morphometric data include;

- Spatial and temporal differences in the incidence of symptoms and animal conditions.
- Identification of specific woylie individuals of interest for comparison with disease datasets that may provide evidence for the potential role of specific diseases in recent woylie declines.
- Assist disease specialists in the interpretation of disease data generated for the same individuals.

A closer examination of data is, however, required in the first instance. For example, the higher prevalence of records of eye and skin conditions in Balban and Winnejup are particularly interesting given the concurrent transition of the Balban woylie population from high to low density. This may provide associative evidence relating to the woylie declines, and/or clues as to the possible cause(s) of the declines. However, before this can be substantiated, the extent of observer differences in the field health checks first needs to be established.

This example, highlights the importance of consistency and quality of data generated for comparative health assessments, between observers and over space and time. The depth of disease and health data being collected from fauna as part of this research is unprecedented in DEC standardised monitoring protocols and the vast majority of fauna research more generally. Nonetheless, it is clear that ongoing critical review is necessary to optimise data quality and collection efficiency. For example, more detailed categorisation and scoring of severity of symptoms and conditions would assist in the improved standardization of reporting, which in turn, assists in the data analysis and interpretation. Adequate training of field staff is also centrally important to quality control and data management, particularly given that these skill sets are, in most cases, new to those that have traditionally been involved in fauna monitoring and research.

The full benefits of the detailed examination of trapped individuals that has been initiated in this research project are yet to be realized. Nonetheless, they are expected to include an increased likelihood in the detection of clues that may assist in the identification of the cause(s) for recent woylie declines, more specifically the role that disease(s) may or may not play, and more generally a deeper understanding of the ecology and biology of the woylie.

5.2.4.2. Disease sampling

The vast majority of samples collected for disease screening were associated with the PCS sites and Upper Warren fauna monitoring transects. Samples were also collected opportunistically from other Western Australian and South Australian sites for comparative purposes.

Samples sizes required to investigate diseases need to be sufficient to provide adequate representation within comparative sites to be able to confidently compare between sites. Furthermore, repeat sampling at these levels are required to investigate temporal changes in prevalence that might be associated with woylie declines. This necessitates having large samples sizes given the need to confidently detect low prevalence rates of pathogens and factors that can still have substantial affects on populations. For example, more than 59 samples are required to detect the presence of an agent with a true prevalence rate of 5% with 95% confidence (SAS). On this basis, despite the sample sizes being practically very large, statistically there may be inference limitations, particularly for the additional non-PCS sites.

5.2.4.3. Radio-collared woylie cohorts

The repeat sampling of woylie individuals over time provides a powerful investigative tool that may provide clues to what might be causing woylie declines at the population level. The radio-collared cohorts provide an especially useful subset of case histories given the additional information available regarding the survival and mortality of these individuals. While the data has now been predominantly organized in preparation for interrogation, the commencement of the development of these case histories remains to be started.

5.2.5. Future work

Field health check data;

- Complete categorisation of symptom descriptions.
- Assess observer differences in reporting of symptoms.
- Investigate differences between sites and temporal changes in the incidence of symptoms and associate the results to differences and changes in woylie populations.
- Make field check data available to collaborating disease investigators for comparison with other disease data.
- Review field health check procedures and data recording.

Disease sampling, once initial disease results are considered and through the co-operation with the WDRC;

1. Review the merits of repeat sampling to provide longitudinal context to disease incidence and prevalence.
2. Review whether sample sizes for sites of interest need increasing to increase the confidence of incidence/prevalence rates for specific diseases of interest.
3. Review whether new disease sampling is merited for specific diseases of interest that may have a role in woylie declines but have not yet been investigated (e.g. urine, oral-pharyngeal swabs, etc).
4. Co-operate with the Murdoch/DEC ARC research project to provide opportunities for sympatric species of medium-sized mammals within the Upper Warren region to be sampled for a similar suite of diseases to develop a more complete understanding of the diseases and parasites within native Western Australian fauna.

Radio-collared cohorts;

1. Complete the collation of all available data to develop full medical case histories of radio-collared individuals.
2. Examine disease and health data of radio-collared individuals to determine if there are any relationships between sites and individuals that died or survived the study period.

5.2.6. Conclusion

Routine field health checks have been incorporated into the standard operating procedures for fauna monitoring in the Upper Warren region. These have been used as the model adopted by the Murdoch/DEC ARC research project into wildlife diseases in WA threatened mammals and provides an opportunity for incorporation into broader Departmental monitoring and research activities, such as *Western Shield*. The field health checks substantially increase the likelihood of the detection of important information potentially relevant to woylie declines and to woylie ecology and biology more generally. The analysis of the data collected to date remains to be completed. The analysis of the disease samples is underway and progress is reported in subsequent sections of this report. The development of medical case-histories for the radio-collared woylie cohorts remains to be started.

5.2.7. Acknowledgements

We would like to thank, i) the WDRC for assistance in the development of the field health check and sampling procedures, ii) the field staff that conducted the health checks and assisted in the collection of samples, and iii) the volunteers for assisting in these activities.

5.3. Clinical

Paul Eden (Associate Veterinarian), Andrea Reiss (Acting Specialist Veterinary Manager), Karen Payne (Veterinarian).

Perth Zoo

Abstract

Perth Zoo Veterinary Department has been contributing to investigations into disease aspects of population declines in woylies (*Bettongia penicillata ogilbyi*) since April 2006. This has involved contribution at a number of levels, including development and co-ordination of clinical sampling protocols, acting as a responder for veterinary management of sick or injured woylies found in the field, on-site participation in trapping programs including collection of diagnostic samples, and contribution of clinical knowledge and expertise to research programs investigating health and disease of woylies. Since becoming involved in this program, seven woylies have been presented to Perth Zoo Veterinary Department with various injuries. Four of these animals were euthanased and sent to Murdoch University for necropsy examination. Three animals were rehabilitated and returned to their original location. Three orphaned woylies were also reported to have developed Metabolic Bone Disease, two of which were euthanased. Perth Zoo veterinarians have also participated in examination of over 150 woylies from field sites in the Upper Warren region and at Karakamia sanctuary. There has not been any conclusive evidence to indicate a particular underlying disease process based on findings of these examinations to date, however this process has contributed valuable information in regards to captive management and rehabilitation of woylies as well as providing relevant samples for other investigation projects in this research program.

5.3.1. Introduction

Perth Zoo was approached by Dr Adrian Wayne from the Department of Environment and Conservation (then Department of Conservation and Land Management) for clinical assistance with the Woylie Conservation Research Project (WCRP) in April 2006. Our primary roles with involvement in this project have been:

- To contribute clinical knowledge and expertise to the Woylie Disease Reference Council as an active member of the council, particularly from the perspective of veterinarians trained and experienced in diseases of wildlife.
- To act as a responder to sick or injured woylies found in the field, with provision of veterinary assistance for these animals and contribution to investigations into potential diseases as a cause of population declines.
- To actively assess animal health and investigate disease during population surveys
- To assist in co-ordination of and provide advice for the sampling of live wild woylies trapped as part of the research program. This has included the development of protocols for the clinical sampling of live woylies sampled in the field and at Perth Zoo (Volume 3), as well as active participation and co-ordination of sampling sessions.

This chapter will discuss findings from the clinical cases presented to the Perth Zoo Veterinary Department since May 2006, as well as examination of animals in the field and clinical issues that have arisen through other channels.

5.3.2. Methods

5.3.2.1. Clinical Cases

Wild woylies showing symptoms of injury or illness were presented to the Perth Zoo Veterinary Department for veterinary attention. Animals with disease or injuries of a known cause (and considered unlikely to contribute to population declines), and which were felt to have potential for return to the wild, were treated and passed on to wildlife rehabilitators for return to the wild (e.g. concussion from trapping trauma). Samples were collected as per the protocol in the WCRP Operations Handbook (Volume 3). Animals with evidence of disease that may have contributed to population declines, where the welfare of the individual was significantly compromised, or that was considered untreatable for return to the wild, were anaesthetized with Isoflurane and oxygen delivered via face mask, examined for health assessment with samples collected as per the WCRP Operations Handbook, and then humanely euthanased with an intravenous overdose of barbiturate. The cadaver and samples were then transferred to Murdoch University for further analysis.

Information was also obtained from external veterinary practices and other sources of information regarding any clinical issues that were reported to Dr Adrian Wayne, by use of phone conversation and email.

5.3.2.2. Field examinations and sampling

In July 2006, Perth Zoo veterinarians Dr Paul Eden and Dr Karen Payne participated in sample collection and physical examination of trapped woylies from the Karakamia Wildlife Sanctuary site near Chidlow, representing an as yet unaffected population. Over 80 animals were sampled and examined on the single day of trapping.

In November 2007 two Perth Zoo vets (Dr Andrea Reiss and Dr Karen Payne) attended the Upper Warren trapping sessions for a total of eight trapping nights and examined approximately 100 animals in the field. Three separate trapping sites were surveyed and included one which had recently undergone a decline (Balban), one which had not yet undergone a decline (Keninup), and one which was thought to be recovering from a previous decline (Warrup). Blood samples were collected from around 80 individuals, and skin samples (under general anaesthesia, performed at mobile field stations) were collected from three individuals, in order to investigate suspected skin disease.

5.3.3. Results

5.3.3.1. Clinical Cases

Six woylies have been presented to Perth Zoo for veterinary attention since May 2006. These cases are summarized in Table 5.3.1. Two woylies were treated for their injuries and returned to the wild – on discussion with various parties (including Dr Adrian Wayne), it was felt that the injuries sustained by these animals were not likely to be contributing to the observed population declines for this species. Four woylies were euthanased and samples collected as described above, and the cadaver and samples sent to Murdoch University for further analysis.

Table 5.3.1. Summary of clinical presentations of sick and injured woylies to Perth Zoo.

PZ ID	Ear Tag	Date of Arrival	Source*	Age	Sex	Wt (g)	Ecto parasites	Clinical summary	Blood	Result	Swab	Other	Fate	MU Path number	Comments
M120636	none (new capture)	12/06/06	K	A	M	858	ticks, lice	Thin,dehydrated(>10%), ulcerated granulating lesions LH foot with evidence of reactive bone on Radiographs	EDTA + serum		wound, cloacal	urine, impression smears of wound	E – sent Murdoch for Necropsy	06-591	
M080742	K1065	8/07/06	K	A	F		ticks, fleas	R eye - Deep corneal stromal abscess with descemetocoele and uveitis	EDTA + serum				Returned		Possible trapping injury? Treated and returned to Karakamia; no fungal elem on in-house cytology
M150743	D03895/ D03896	15/07/06	UW	A	F		ticks	Trapping injury - hindlimb paresis; spinal bruising?; corenal ulceration R eye	EDTA + Serum			Urine; faeces	Returned		Treated and returned to wi fully furred pouch young expelled at trapping and gi to carer for hand raising
M2908117	K1129	29/08/06	K	A	M	1004	lice, fleas	Large maggot infested draining abscess/granuloma R side neck; reasonable body condition	EDTA + Serum		Oral swab	Urine; faeces	E – sent Murdoch for Necropsy	06-879	
M170529	K1008	17/05/07	K	A	M	840		sig wt loss and neurological/ocular symptoms, azotemic on bloods.	EDTA + serum	NSF	Oral swab	Urine free catch	E – sent Murdoch for Necropsy	07-658	Aged animal? given degre tooth wear
M210532	none	21/05/07	K	A	M		yes - type unknown	open wound and swelling around L hock, conjunctivitis R eye	EDTA + serum		Oral swab; swabs from L hock	urine	E – sent Murdoch for Necropsy	07-659	L hock - heavy mixed grow Pasteurella, Aeromonas hydrophila, Klebsiella oxyt Conjunctiva - mod mixed g coag neg Staph, Corynebacterium bacilli
M251063	DO1553/ DO1554	25/10/07	UW	S	M		ticks, lice	Found w odd behaviour - possible road trauma?? Heavy ectoparasite infestation and anaemic (possibly linked). Improved w symptomatic therapy.	EDTA+ serum	anaemia	Oral swab	Faeces	Returned to UW for release		

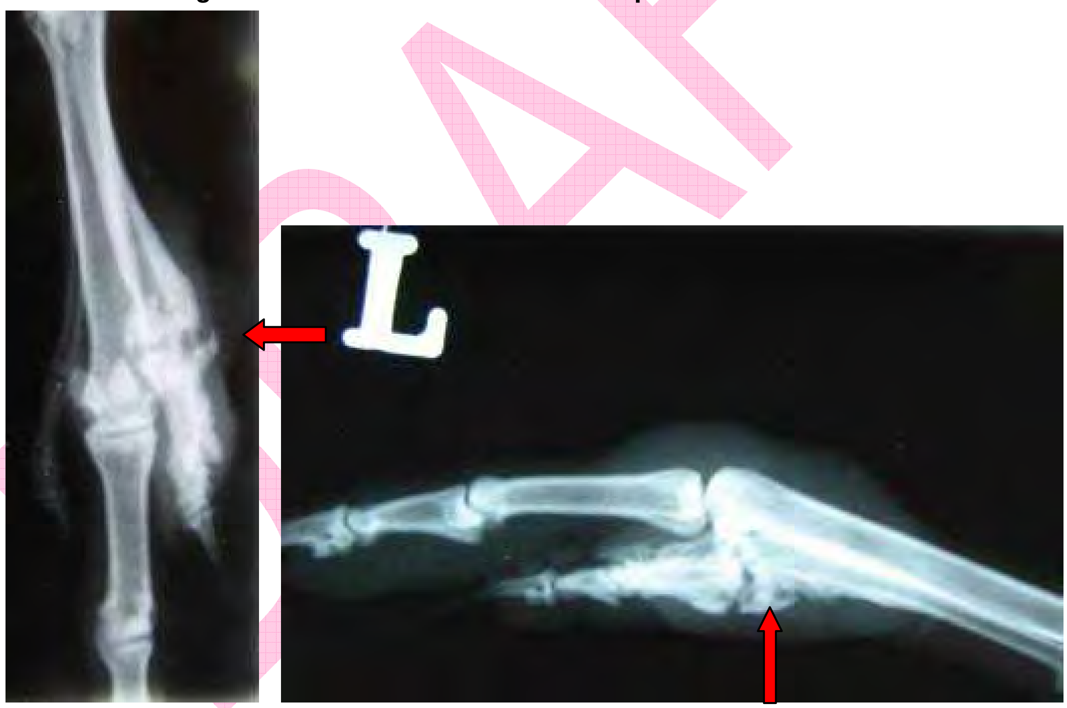
*K=Karakamia, UW =Upper Warren

DRAFT

Woylie M120636 (no tag id)

This animal originated from Karakamia and presented with swelling and open granulating wounds to its left hind foot (Figure 5.3.1). It was also thought to be in thin condition, with a body weight of 858g, and was dehydrated. Radiographs of the affected region revealed evidence of underlying bone pathology (Figures 5.3.2 a&b). This animal was euthanased as per above discussions.

Figure 5.3.1 Skin lesions on outer aspect of left hind foot.



Figures 5.3.2. a & b Radiographic lesions of left hind foot. Note the extra bone proliferation and evidence of bone lysis and joint deformity (arrow).

a) Dorsal view. b) Lateral view.

Woylie M080742 (K1065)

This animal was observed to have a problem with its right eye during a sampling session held at Karakamia in July 2006. Closer inspection of the eye revealed a deep abscess of the cornea of the right eye and secondary uveitis. The lesion was thought to have originated through trauma (possibly from trapping), and it was elected to treat this animal and return it to Karakamia when recovered. Treatment involved daily application of topical antibiotic and anti-inflammatory eye medication, subconjunctival injections of Gentamicin, plus systemic anti-inflammatories. The animal was also anaesthetized on a regular basis for monitoring of the eye. The abscess responded to treatment, although a scar had developed on the surface of the eye as a consequence of the abscess. The impact of this on the animal's vision was discussed with Karakamia and Adrian Wayne, and it was decided to return this animal to Karakamia where the outcome of this animal's return was more likely to be known. Following veterinary treatment this animal was sent on to Chidlow Marsupial Hospital for further rehabilitation prior to release.

Woylie M150743 (D03895/D03896)

This woylie was presented due to an acute spinal injury sustained during trapping for population surveillance. Symptoms included paresis of the hind limbs, with reduced activity and reflexes, as well as general depression. It originated from the Upper Warren region. Examination under anaesthesia revealed an area of deep bruising over the lumbar spine. Radiographs revealed there was no evidence of a spinal fracture or injury. This animal was given supportive therapy with rest and systemic anti-inflammatories. It recovered from its injuries and was returned to Upper Warren.

Woylie M2908117 (K1129)

This animal was presented from Karakamia with a large ulcerated swelling over the right side of the neck. The swelling was infested with fly larvae. This animal was in reasonable body condition at presentation. Upon discussion with relevant parties, this animal was euthanased as described above.

Woylie M170529 (K1008)

This animal was presented from Karakamia with evidence of severe visual deficits in both eyes. It was also found to be in thin condition. Blood tests revealed evidence of renal failure. Ocular examination did not reveal evidence of retinal detachment, however did reveal evidence of retinal degeneration. This animal was euthanased as previously described.

Woylie M210532 (no ear tag)

This animal presented from Karakamia with swelling and an open wound over the left hock joint, as well as an injury to the right eye. Examination under anaesthesia revealed the animal to be in suboptimal body condition, an open fracture of the left hock and reddening of the conjunctiva of the right eye. Radiographs confirmed the presence of a fracture involving the left hock joint (Figure 5.3.3). These injuries were though to be a few days old and were likely to be a result of trauma. Due to the severity of the injury to the left hock, this animal was euthanased as described above.

Figure 5.3.3 Fractured hock (arrow).

Woylie M251063 (DO1553/1554)

This woylie was found in the Upper Warren region. It was showing signs of unusual behaviour, including subtle ataxia, and was poorly responsive to approach by people. Examination showed a heavy infection with ectoparasites, with evidence of anaemia on haematology. There was also a slightly reduced responsiveness to the left pupil, and biochemistry revealed evidence of muscle injury, consistent with trauma. No other abnormalities were found. This animal underwent supportive treatment at Perth Zoo Vet Department, including ectoparasite control, and responded well to this. The animal was returned to the Upper Warren area for release where it was found.

5.3.3.2. Metabolic Bone Disease

Three case of Metabolic Bone Disease were reported from Busselton Veterinary Clinic (Dr Richard Lucas). The three woylies involved were all orphaned pouch-young that were hand reared by a wildlife rehabilitator with substantial experience with woylies. The three affected animals showed symptoms of lethargy and reduced activity. Radiographs revealed thinning of the bone cortices in all three animals, whilst two animals had pathologic fractures of the distal femur. These two animals were euthanased on welfare grounds. The remaining affected animal was treated with calcium supplementation and continues to be in good health. This animal is currently housed at Yelverton Eco-retreat in a predator proof enclosure where it can be monitored regularly.

5.3.3.3. Field investigation of skin disease

Clinical signs of skin disease (hair loss, skin thickening, redness and scabbing) had been noted in Woylies during population monitoring earlier in 2007. Biopsy samples were collected from three individuals with signs of skin disease during trapping sessions at Balban (1) and Keninup (2). A biopsy was also taken of woylie *M251063 DO1553, 1554* prior to its release to serve as a reference for “normal”. Histopathological examination of the skin tissues showed either normal skin or mild non-specific inflammatory changes. Occasional larvae of an unidentified parasite were seen within the keratinized layers of both normal and inflamed skin samples in two animals. Microbiological culture of the skin samples showed only normal skin flora, or organisms which might be found as opportunistic agents.

5.3.4. Discussion

Based on the limited number of sick or injured woylies presented to the Perth Zoo Veterinary Department to date, there has been no clear indication as to an underlying disease process that may be contributing to the declining woylie populations in southwestern Australia. Three woylies presented with lesions that would be consistent with an initial traumatic injury that was secondarily infected (i.e. not thought to be related to observed woylie declines). The woylie that presented with renal failure and visual deficits is an interesting case. It is possible the visual deficits could be related to retinal detachment from increased blood pressure associated with renal failure, however there was no evidence of retinal detachment on examination of the eyes. Many factors can result in renal failure, including diet, exposure to toxins, age-related degeneration, and infectious diseases. This animal also originated from Karakamia, where declines in woylie populations have not been observed to date. It is therefore likely this was a singular occurrence of this problem, unrelated to population declines (particularly given that the animal came for Karakamia where woylie declines have not been observed).

Metabolic bone disease (MBD) is largely a disease of captive animals, and is mostly caused by nutritional and husbandry issues. In particular, diets deficient in calcium or with a calcium/phosphorus imbalance, or the lack of exposure to UV light for Vitamin D3 activation, are commonly implicated in the development of this complex disease. Discussions have been held between the various parties involved in an attempt to identify an underlying cause for these three animals, however no underlying issue was identified. This disease is of significance in relation to captive management of woylies. Its significance in relation to population declines is likely to be low, however MBD has been reported in wild populations of mammals in other parts of the world (various deer and rodent species, Ullrey, 2003; beluga whales, Mikaelian *et al*., 1999), and continued monitoring for this condition, including routine radiographs of woylies presented for clinical assessment, will be undertaken.

5.3.5. Future work/Conclusion

This process has provided a valuable contribution to the knowledge of the health and disease status of woylies by monitoring of clinical presentations of sick or injured woylies. The health assessment and sampling protocol for these woylies has been working well so far, however it will be refined to include routine radiography for all sick or injured woylies presented to the Perth Zoo Veterinary Department. It has also provided the opportunity to collect samples that are utilized in other investigations as part of this research program. It has also contributed to furthering the development of techniques required for rehabilitation of woylies for return to the wild.

5.3.6. References

Mikaelian, I. Qualls CW Jr, De Guise S, Whaley MW, Martineau D (1999), Bone fluoride concentrations in beluga whales from Canada, *J Wildlife Disease* **35(2):** 356-360.

Ullrey DE (2003), Ch 80: Metabolic Bone Disease, In ME Fowler and RE Miller (eds), *Zoo and Wildlife Medicine 5th ed*, Saunders, Missouri.

5.3.7. Appendices

Refer to relevant sections in WCRP Operations Handbook (Volume 3) for: Field Clinical Examination Checklist for Woylies and Perth Zoo Clinical Examination Checklist for Woylies.

Volume 2 Appendix 4 – Metabolic Bone Disease of Woylies.

5.4. Pathology

Graeme Knowles[1], Adrian Wayne[2], Paul Eden[3], Nicky Marlow[2], Phil Nicholls[1]

[1]Murdoch University
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[3]Perth Zoo

Abstract

From September 2005 to May 2007 officers from Department of Environment and Conservation and staff from Australian Wildlife Conservancy, submitted woylies found dead in monitored wild populations in southwestern Australia and Karakamia Wildlife Sanctuary (Chidlow, WA), respectively, to the Murdoch University pathology department. Moribund animals were sent to Perth Zoo. Field data associated with each animal submitted, for example site description, weather, evidence of predators (scats, tracks, etc), and state of the remains, was recorded by the submitters.

Thirty one woylies were submitted for necropsy at Murdoch University. Thirteen were males, 16 female and two were unknown (because only limited body parts were found in the field). All animals submitted were adults. One female adult had a pouch-young.

Veterinary pathologists from School of Veterinary and Biomedical Sciences, Murdoch University conducted all necropsies. The causes of death included;

- skeletal fractures/ marked haemorrhage (7) (mostly suspected road accidents)
- skeletal fractures/haemorrhage with puncture (bite) wounds (6) (predation)
- cardiomyopathy / skeletal muscle myopathy (2)

In 11 cases the cause of death could not be determined grossly or by histopathology. All but one of the animals, whose cause of death was unknown, was in good body condition. For this reason, in conjunction with the unremarkable gross and microscopic findings, an acute process rather than chronic disease was the likely cause for the unknown mortalities.

Four animals presented moribund with marked focal dermal lesions and one had septic arthritis. Culture results indicated these were isolated cases of non-transmissible diseases.

5.4.1. Introduction

There has been a sudden decline in the woylie population in southwestern Australia within the past 2 years. To investigate cause for woylie decline Department of Environment and Conservation coordinated (through the Woylie Conservation Research Project) three areas of research; predation, resources and disease.

DEC requested Murdoch University and Perth Zoo to investigate disease factors that could contribute to the decline. Methods used to investigate disease factors included necropsy of animals found dead as well as assessment of live captured animals (blood taken for clinical pathology, faecal analysis for endoparasites, external parasites).

5.4.2. Methods

From September 2005 to May 2007 officers from Department of Environment and Conservation and staff from Australian Wildlife Conservancy, submitted woylies found dead in monitored wild populations in southwestern Australia and Karakamia Wildlife Sanctuary (Chidlow, WA), respectively, to the Murdoch University pathology department. Moribund animals were sent to Perth Zoo, and examined clinically. They were all euthanized for humane reasons and submitted to Murdoch University for necropsy. Field data including, for example site description, weather, evidence of predators (scats, tracks, etc), and state of the remains, was recorded by the submitters.

Staff veterinary pathologists from the School of veterinary and biomedical science, Murdoch University, conducted all necropsies. Histopathology (using haematoxylin and eosin stains) was conducted on tissues from all animals submitted (unless the carcase was markedly degenerate). If indicated (e.g. gross changes indicating inflammatory or necrotic processes) tissues were sent for bacterial culture. Intestinal contents were taken for parasitological assay (McMaster method). Stomach contents were taken to assess diet. Ethanol fixed heart, skeletal muscle and brain was set aside for PCR assay for *Toxoplasma* (Bretagne, 1993).

In addition, multiple organ samples (including liver, spleen, kidney, heart, and lung) were frozen and oropharyngeal swabs held frozen in virus transport media. These were kept for possible future molecular assays or virus culture to further investigate the cause of death where gross and microscopic findings were unremarkable.

Swabs of bite wounds (on the collar), from one of the animals which had evidence of trauma and bite wounds at necropsy, were sent for PCR analysis to identify the predator species. The assay used mtDNA primers for the control region and cytochrome b gene for species identification (P Spencer personal communications).

5.4.3. Results

5.4.3.1. Gender and age

Thirty one woylies were submitted for necropsy at Murdoch University. Thirteen were males, 16 female and two were unknown (because only limited body parts were found in the field). All animals submitted were adults. One female adult had a pouch-young.

5.4.3.2. Causes of death and morbidity

The cause of death was concluded most commonly from gross assessment.

Trauma was the most common cause of death identified. No other findings were evident in these animals to indicate intercurrent or chronic disease. That is, there were no factors found that could have predisposed to predation or other forms of trauma.

Table 5.4.1. Overall assessment of cause of death and morbidity of woylies submitted for necropsy.

Number of animals	Cause of death	Cause of morbidity
7	Skeletal fractures/ marked haemorrhage	
6	Skeletal fractures/haemorrhage with puncture (bite) wounds	
2	Cardiomyopathy / myopathy	
11	Unknown	
4		Chronic focal dermatitis
1		Chronic septic arthritis
31	Total	

The majority of animals with skeletal fractures/ marked haemorrhage were found near the road suggesting the cause of death was road accidents.

The one case of cardiomyopathy showed microscopic changes strongly suggestive of exertional cardiomyopathy. This occurs following excessive adrenaline release and marked sudden demand on cardiac function. The skeletal myopathy was unusual with regenerative changes evident only in the tongue and oesophagus.

In 11 cases the cause of death could not be determined grossly or by histopathology. All but one of the animals, whose cause of death was unknown, was in good body condition. For this reason, in conjunction with the unremarkable gross and microscopic findings, an acute process rather than chronic disease was the likely cause for the unknown mortalities.

Four animals presented moribund with marked focal dermal lesions and one had septic arthritis. The bacteria isolated from the skin lesions included *Bacillus cereus*, *Staphylococcus* spp, *E. coli* and *Pasteurella* spp. Mixed bacterial growth was isolated from the septic arthritis lesion. The culture results indicated these were isolated cases which were due non-transmissible.

The location where the animal was found was recorded and this has been tabled below against the cause of death or morbidity

Table 5.4.2. Location and cause of death and morbidity of woylies submitted for necropsy.

	Cause of death				Cause of morbidity	
Site	Skeletal fractures/ marked haemorrhage	Predation	Cardiomyopathy / myopathy	Unknown	Chronic septic arthritis	Focal dermatitis / osteomyelitis
Keninup	2	1				
Balban	1					
Karakamia	1			4	1	4
Chariup			1			
Manjimup	1					
Batalling	1					
Dryandra		3		3		
Tutanning	1	2		4		
Wellard			1			

5.4.3.3. Parasitology

Intestinal content or faeces were not collected from all submitted animals. If the animal remains were markedly degenerate samples were not taken.

Table 5.4.3. Nematode or eggs identified from woylies submitted for necropsy.

	Strongyle eggs	Lungworm larvae	Adult protostrongyle woylie	Unknown or degenerate larvae	Adult parastrongylus bettongia	No nematode or eggs seen
Number of animals	5	1	1	2	1	13

5.4.3.4. *Toxoplasma* PCR

To date, only three woylie tissue samples were tested for *Toxoplasma* DNA. This was using PCR outlined in (Bretagne *et al*., 1993). All samples were negative.

5.4.3.5. PCR analysis of bite wound swabs

Swabs of the bites on the tracking collar of one animal with multiple bite wounds and haemorrhage into the chest (supporting predation as the cause of death) were submitted for PCR but the results were unrewarding and the species of the predator could not be confirmed.

5.4.4. Discussion

The cause of death was determined in 15 out of 26 cases submitted for necropsy (5 other animals were euthanized due to chronic dermal lesions or septic arthritis). Most commonly the cause of death was concluded based on gross findings. Trauma was the most common cause of death. No other findings were evident in these animals to indicate intercurrent or chronic disease. That is, there were no factors found grossly or microscopically that could have predisposed to predation.

In 11 cases the cause of death could not be determined grossly or by histopathology. All but one of the animals, whose cause of death was unknown, was in good body condition. For this reason, in conjunction with the unremarkable gross and microscopic findings, an acute process rather than chronic disease was the likely cause for the unknown mortalities. Surveys of sudden death (unexpected death in apparently healthy animals) of companion animals and equines (including necropsy and histopathological assessment) often have a significant proportion of cases of unknown causes of death varying from 30% to 60% (Brown, 1988; Knowles, 2006).

Bloods taken from collared animals which were later found dead will be analysed and the haematology assessed to see if this will further clarify a cause of death in those cases of unknown sudden death.

Two groups of differential diagnoses for sudden death in woylies with unremarkable gross and microscopic findings may be considered. These are causes

a) which relate to individual deaths for example defects in cardiac conductivity, snake bite, lightning strike, hypothermia

b) infectious / toxic causes affecting a common population e.g. botulism, acute plant toxicoses (e.g. plants containing cardiac glycosides, fluoroacetate compounds).

The interests of this study are in the latter group of differential diagnoses i.e. diseases affecting a population group. Botulism is uncommonly reported in marsupials. Testing of fresh feed or serology (blood sample) is required to assess for botulinum toxin. These samples were not available. Plant toxicology relating to native marsupials is limited. Assessment of fresh feed is required. Of course an unknown infectious or toxic agent cannot be excluded as the cause of the sudden death. Serology will be undertaken from trapped woylies for orbivirus, herpesvirus and flavivirus. Please note that chorioretinitis (orbivirus infection in kangaroos), epithelial or hepatic necrosis (herpesvirus) or encephalitis (flavivirus) which are commonly microscopic findings associated with these viruses were not noted in the cases examined.

The case of cardiomyopathy showed microscopic changes strongly suggestive of adrenaline induced cardiomyopathy. This occurs following excessive adrenaline release (due to severe acute stress) and marked sudden demand on cardiac function. The second case of myopathy displayed chronic skeletal muscle regeneration secondary to muscle degeneration but only in selective sites (tongue and oesophagus). A cause could only be speculated based on extrapolation from ruminants. In ruminants causes of myopathy include selenium and vitamin E deficiency, ingestion of toxins such as gossypol, *Cassia* spp or prolonged intensive anaerobic exertion (Van Vleet and Valentine, 2007).

Those woylies which had intestinal parasites were in reasonable body condition and there were no significant gross or microscopic pathological lesions in the intestinal tract. For these reasons the endoparasites identified were incidental findings and did not contribute to the cause of death or morbidity.

5.4.5. Future work

The haematology of collared animals, which were later found dead (and necropsied), will be used to further investigate possible predisposing factors to morbidity and death. Serological surveys of blood from trapped woylies will be undertaken in the next month at UWA. Serological assays for orbivirus, herpesvirus and flavivirus will be undertaken. Based upon serological results, PCR analysis or virus culture of stored samples may be considered on those animals that had inconclusive necropsy findings. Assessment of stomach contents from dead woylies for seeds of known toxic plants will also be considered.

5.4.6. Conclusion

This was a small survey of dead and moribund woylies (31 animals submitted in total). There was no predominant cause evident for the death of woylies submitted. Road accidents and predation were causes of death. DEC staff, having knowledge of the historic records of woylie populations, will interpret the importance of these factors. Further analysis of haematological data (for collared / tracked woylies) is pending. This will be particularly helpful for those cases of sudden death of unknown cause. Further molecular assays based on serological surveys to assess for exposure of the woylie population to orbivirus, herpesvirus and flavivirus infection will be considered for the stored tissues from the animals with unresolved causes of death.

5.4.7. References

Bretagne, S., J. M. Costa, *et al.* (1993) "Detection of *Toxoplasma* gondii by competitive DNA amplification of bronchoalveolar lavage samples" J Infect Dis 168(6): 1585-8

Brown CM et al. (1988) Equine vet J 20, 99

Knowles GW et al. (2006) ASVP Ann Conf 2006

Van Vleet JF and Valentine BA Muscle and tendon p 185-279 in Pathology of domestic animals vol 1 Ed MG Maxie 2007

5.4.8. Acknowledgements:

The dead woylies were submitted to Murdoch University by staff of the Department of Environment and Conservation, Australian Wildlife Conservancy and Perth Zoo. The necropsies were undertaken by pathologists at Murdoch University; Dr Graeme Knowles, Dr Philip Nicholls, Dr Mandy O'Hara, Dr Sandy McLachlan, Dr Lucy Woolford and Dr Mark Bennett. Parasitology was undertaken at Murdoch University by Aileen Eliott and Russell Hobbs, School of Veterinary and Biomedical Sciences, Murdoch University. PCR analysis for *Toxoplasma* was done by Dr Nevi Parameswaran, School of Veterinary and Biomedical Sciences, Murdoch University and PCR analysis of bite swabs for species identification was completed by Dr Peter Spencer, School of biology, Murdoch University.

5.5. Haematology

5.5.1. Editor's Note

Considerable haematological work has been conducted as part of the WCRP. Unfortunately, much of it is not reported here. With the recent departure of Dr Phillip Clark from Murdoch University, his capacity to remain involved in the WCRP has also ceased. Dr Clark's contribution to the WCRP during 2006 and much of 2007 was highly valuable and integral to diagnostic disease investigations into the woylie declines, for which I am deeply grateful. His involvement and expertise are very much missed but we wish him the very best in is new persuits. Efforts are currently underway to address the resulting vacancy in haematological expertise.

Below is a copy of the haematology progress report extract from the Mesopredator Review, November 2006 by Phillip Clark. Further elaboration is provided by a short 2007 update courtesy of Paul Eden.

5.5.2. Haematology progress report extract from the Mesopredator Review, November 2006

Phillip Clark

Murdoch University

Blood samples were analysed from approximately 168 woylies from a number of locations. For the purpose of this report, the animals have been analysed as from 'Perup' or 'Karakamia'. Further analysis will become possible when animal identification data from each of the Perup transects becomes available. Overall, few (4 from Perup, 0 from Karakamia) animals exhibited haematological evidence of a distinct disease process (such as anaemia or inflammation). Data from these animals was excluded from the current analysis. The current analysis was generated using data collected from 130 animals (105 animals from Perup Data and from 25 animals from Karakamia). The dataset was incomplete in approximately 20 animals.

Notably, two haemoparasites were recognized. These were an intra-erythrocytic piroplasm (morphologically consistent with *Theileria penicillata* sp. nov., Clark and Spencer *in press*) and a previously unreported species of *Trypanosma*.

Data were obtained from 25 animals from Karakamia. Of these, 95% (20 animals) had piroplasms visible in blood smears by light microscopy. However, trypanosomes were not visible by light microscopy in any of the samples examined.

Data was obtained from 105 animals from Perup, at a number of trapping transects. Piroplasms were visible in blood smears from 83% (87 animals) of the animals trapped, and *Trypanosoma* sp in 43% (45 animals).

Further work is being undertaken, using molecular biology methods to confirm the status/prevalence of the haemoparasites and to assess the phylogenetic characteristics of these organisms.

Statistical analysis

Animals that were considered to be unwell were excluded from the statistical analysis.

A Kolmagorov-Smirnof goodness-of-fit test was used to determine whether each variable was normally distributed for each population. All variables except band neutrophil concentration and basophil concentration were normally distributed.

For each analytes, a one-way ANOVA was used to compare the means of the two populations (Table 5.5.1). The Perup population had significantly greater total white blood cell concentrations ($p<0.001$), lymphocyte concentrations ($p<0.001$), monocyte concentrations ($p=0.002$) and eosinophil concentrations ($p=0.007$) than the Karakamia population. The Karakamia population had significantly greater MCH ($p<0.001$) and MCHC ($p<0.001$) than the Perup population.

Despite the observed statistical differences in the leukocytes, these do not clearly identify a distinct disease process and may be due to confounding influences on the leukocyte profile (such as ‘stress’ or excitement).

Table 5.5.1. Summary of analyte values for Perup [Upper Warren region] and Karakamia Wildlife Sanctuary.

Variable	Karakamia		Perup		p
	Mean	Range	Mean	Range	
WBC	3.44	1.5-7.8	5.35	1.3-11.5	<0.001
Lymph	1.53	0.4-4.1	2.45	0.3-7.8	<0.001
Mono	0.083	0.0-0.2	0.15	0.0-0.6	0.002
Eos	0.05	0.0-0.4	0.17	0.0-1.0	0.007
MCH	15.34	13.3-19.9	14.23	11.1-19.9	<0.001
MCHC	318	287-367	300	267-406	<0.001

5.5.3. Haematology update for 2007 in brief

Paul Eden

Perth Zoo

5.5.3.1. Introduction

This section follows on from ongoing work continued from a report by Dr Phil Clark (Murdoch University) from 2006.

5.5.3.2. Methods

Blood samples were collected from woylies following techniques as described in the WCRP Operations Handbook (Volume 3), and were examined using the standard haematological practices of Murdoch University Veterinary Hospital Clinical Pathology Laboratory. Samples were analysed by Dr Phil Clark and his associates. Samples were assessed for standard haematological parameters – packed cell volume (PCV, %), red blood cell count (RBC, cells $x10^{12}$/ml), haemoglobin concentration (Hb, mg/dl), white blood cell count (WCC, cells $x10^{9}$/ml), differential white blood cell count (heterophils, eosinophils, basophils, monocytes, lymphocytes, cells $x10^{9}$/ml) and platelets. Morphology of cells, including examination for red blood cell parasites, was also assessed. Samples examined included whole blood mixed with anticoagulant (EDTA) and air dried blood smears stained with Wright’s/Giemsa stain.

5.5.3.3. Results

A total of 222 samples were analysed for haematology over the last twelve months, from samples collected in the Upper Warren region, PCS sites, Dryandra, Tutanning, Batalling and South Australia. (i.e. grand total of 511 samples from March 2006 to December 2007). Reference range information has previously been reported from a limited number of specimens by Dr Phil Clark (see Section 5.5.2. Haemotology Report 2006). This more recent data will be added to the haematology database and analysed in the near future to contribute to the strength of this reference information.

Further investigations regarding haemoparasites has been undertaken and is discussed elsewhere in this report.

5.5.3.4. Discussion

Reference haematological ranges are of value to investigate for evidence of clinical illness in sick and injured woylies, as well as to investigate possible subclinical effects of disease, however reference ranges are only of significant value in relation to the amount and quality data used to create them. Further analysis of haematological data to include more recent data will be undertaken in the near future to strengthen the relevance of the current reference ranges.

5.5.3.5. Future work

Further analysis of data to assess for significant variation in haematological parameters with demographic and health status variables should be undertaken to further investigate for changes associated with disease, age, gender and so on. It is felt that sufficient haematological data is now available from the perspective of developing a sound reference range for this species, and for assessing variation in relation to demographics, location etc. Further analysis of haematological information in relation to health status to investigate for potential subclinical effects of pathogens studied thus far would also be valuable to undertake.

5.5.3.6. Conclusion

A reference range for haematological parameters for woylies has previously been developed based on a limited number of samples (Section 5.5.2. Haemotology Report 2006). Further data analysis to include more recently obtained data will be undertaken to strengthen this reference information. Further investigations regarding haemoparasites have been undertaken and are discussed elsewhere in this report.

5.6. Trypanosomes

Andrew Smith and Susana Averis

Murdoch University.

Abstract

Trypanosomes of the order Kinetoplastida are introduced within a framework of their biological diversity and ecological impact. A morphologically distinct trypanosome species was detected by light microscopy at relatively high prevalence level of ~ 40% within the declining woylie population of the Upper Warren region. Initial investigation by light microscopy revealed no trypanosomes within the stable and fenced woylie population within the Karakamia Wildlife Sanctuary (operated by Australian Wildlife Conservancy). Further investigation employing molecular techniques revealed the trypanosome within the Upper Warren area to be novel based on analysis of the 18SrRNA gene, and also to be present within the Karakamia population regardless of initial microscopy results. The potential for trypanosomes to negatively impact on host fitness, either as a singular infection whilst the host experiences additional environmental stress, or as part of a concomitant infection together with *Toxoplasma gondii*, is discussed.

5.6.1. Introduction

Protozoan trypanosomes of the order Kinetoplastida are blood parasites primarily of insects but are known to infect a wide range of mammals, birds, reptiles, amphibians and fish. Some species, such as *T. congolense* and *T. vivax* of cattle are economically significant in many developing nations, with infection resulting in death or reduced output and reproductive capacity through emaciation and anaemia. Infection by the trypanosomes *T. gambiense* and *T. rhodesiense* lead to human trypanosomiasis, otherwise known as sleeping sickness in Africa, whereas infection with *T. cruzi* in South America is known as Chagas disease. All three forms of trypanosomiasis result in chronic infections that can be fatal if not properly treated. In all the above cases, the principal route of infection is through an obligate intermediate host or insect vector such as the tsetse fly in Africa and triatomid bugs in South America.

Many wildlife species are commonly infected with endemic or host-specific trypanosome species with little or no apparent pathological effects. However, infection with novel species often has fatal or significantly debilitating effects. For example, *T. lewisi*, a trypanosome associated with the black rat (*Rattus rattus*) has been implicated in the decline of native rodent species along an invasion front in Madagascar (Laakkonen, 2002; Laakkonen *et al.,* 2003). Pickering and Norris (1996) have also implicated the arrival of *R. rattus* onto Christmas Island and the subsequent spread of *T. lewisi* in the decline and extinction of the endemic rat *R. macleari*. Experimental infections involving mice and voles have resulted in much faster and significantly higher parasitemia rates when non-host specific trypanosomes were inoculated compared to what was observed during infection with a host specific trypanosome species (Maragi *et al.,* 1989). There is also strong evidence to suggest that virulence in trypanosome infection is in part host-condition dependent, with individuals in poor condition as a result of concomitant infections, stress due to resource shortage or even low genetic diversity, more likely to suffer negative effects than healthy individuals (Brown *et al.,* 2000). Furthermore, concomitant infections have also been shown to be affected, with rats experimentally infected with *T. lewisi* showing higher levels of *Toxoplasma gondii* tachyzoites at periods of four and seven days post infection than uninfected control animals (Guerrero *et al.,* 1997; Cox, 2001).

In the present context, trypanosomes are under consideration as a contributory factor in the recent declines of the woylie (*Bettongia penicillata*). There are several factors that suggest a possible role for trypanosomes including reduced survival and increased mortality rather than emigration or poor recruitment as the likely primary mechanisms for population decline. It is also possible that some underlying factors may be increasing the vulnerability of individuals to predation (Chapter 2 UW Fauna Monitoring and Section 4.3 Survival and mortality). In addition, trypanosome infection may be compounding the effects of concomitant infections as mentioned above. It is particularly relevant to note the synergistic effects of trypanosomes when in combination with *Toxoplasma*, a highly pathogenic infection spread primarily by cats and which has been confirmed within the Upper Warren woylie populations (Section 5.7 *Toxoplasma*). The spread of a novel trypanosome species must also be considered in any investigation. Trypanosome vectors of small mammals are generally fleas or ticks or some other blood-sucking insect such as mosquitoes or biting flies. The possibility exists that a non-native trypanosome, possibly originating from a non-native invasive host species such as a cat, fox, rodent or pig etc, has become established within the woylie ectoparasite fauna of the region. It is also possible that a novel ectoparasite vector has jumped host and is now maintaining a novel trypanosome parasite-host cycle within the woylie population.

5.6.2. Methods

Light microscopy has traditionally been used to determine the presence, absence and relative morphology of trypanosomes in appropriately stained blood smears. However, the recent trend has been to adopt more sensitive and specific molecular methods based on DNA amplification by PCR (polymerase chain reaction) to determine presence or absence of infection, and DNA sequencing and genotyping for characterisation and identification to species level. The gene targeted for the present study was a variable region of the trypanosome 18SrRNA gene, with a full description of the techniques used, together with primer details given by Smith *et al.* (2005).

5.6.3. Results

Light microscopy revealed an overall trypanosome prevalence of 43% (n = 45) in a sample of 105 woylies from the Upper Warren, and zero prevalence in a sample of 25 woylies from the Australian Wildlife Conservancy-operated Karakamia population. However, amplification of the small ribosomal RNA subunit (18SrRNA) gene resulted in the detection of two trypanosome-infected animals out of a subset of six individuals re-tested from the Karakamia population that was previously assumed to be trypanosome-free (Figure. 5.6.1).

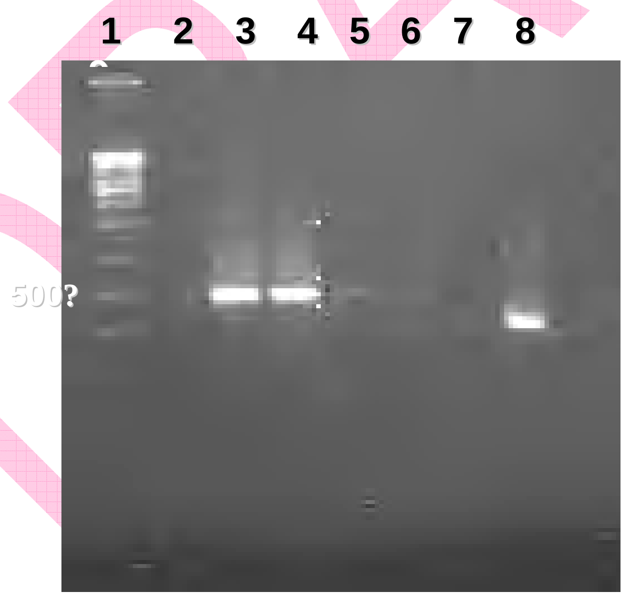

Figure 5.6.1. Trypanosome DNA amplification using primers specific to the 18SrRNA gene. Lane 1: molecular marker 1KB; lanes 2-7: Woylie samples from the Karakamia population; Lane 8: positive control.

Sequencing of the positive samples from Karakamia and Upper Warren suggests they are more closely related to each other than any other trypanosome species in the database, but also closely related to a trypanosome species found in a wombat and in a potoroo (T. sp P63) (Hamilton *et al.*, 2005). Another novel trypanosome species isolated from a chuditch (*Dasyurus geoffroii*) from

Julimar, and most closely related to *T. bennetti* found originally in a kestrel in North America and prevalent in birds, has been included in the phylogenetic tree in Figure 5.7.2. Sequencing of the amplified trypanosome DNA was carried out using NCBI (National Centre for Biotechnology Information) BLAST (Basic Local Alignment Search Tool) programme. The phylogenetic tree was constructed using the programme Mega 4.0, neighbour-joining, 1000 bootstrap with default parameters.

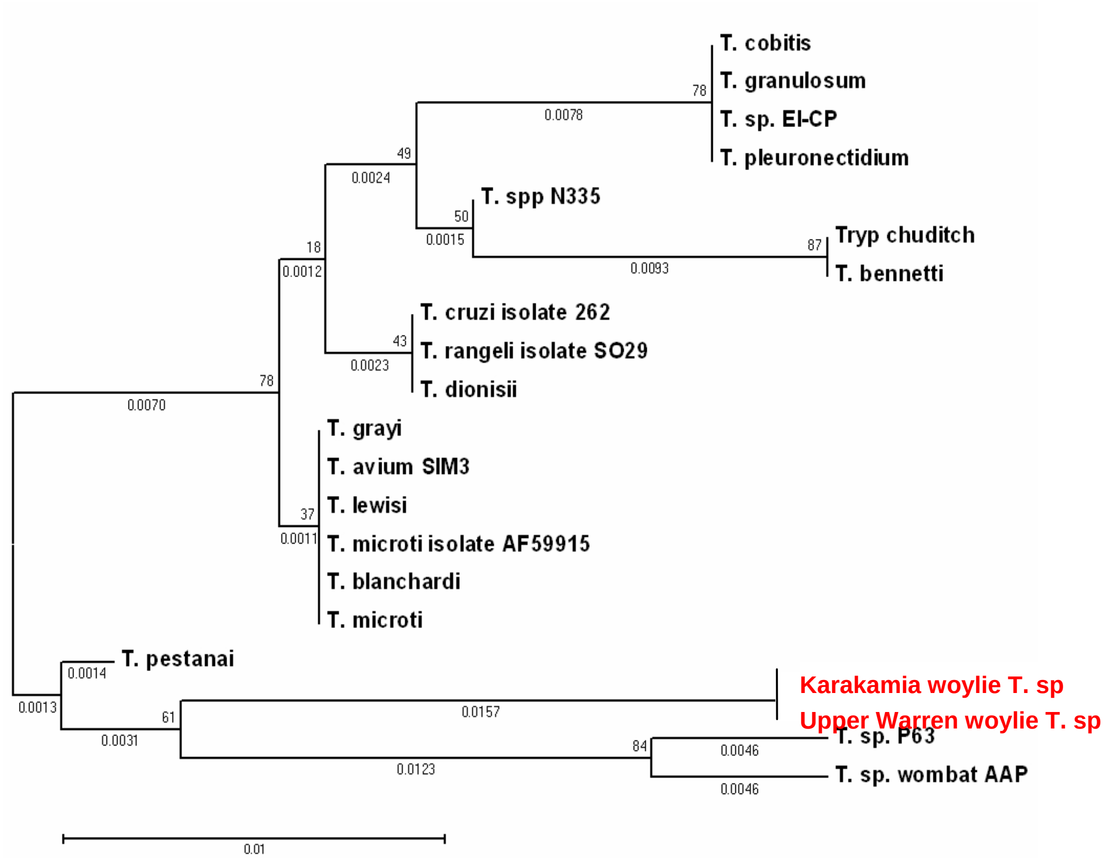

Figure 5.6.2. Phylogenetic tree based on bootstrap analysis of the 18SrRNA gene from 20 trypanosome sequences. Branch numbers represent bootstrap values. The clade that includes the Karakamia woylie trypanosome also includes trypanosomes found in other native species. The trypanosome found in a chuditch sample falls in a clade representing trypanosome species commonly found in birds.

Out of a further subset of five previously untested samples from the Upper Warren population four were found to be positive for trypanosome infection by PCR (Figure 5.6.3). We are currently in the process of DNA cloning and further sequence analysis to determine the genotypes of the trypanosome species present within each of the populations.

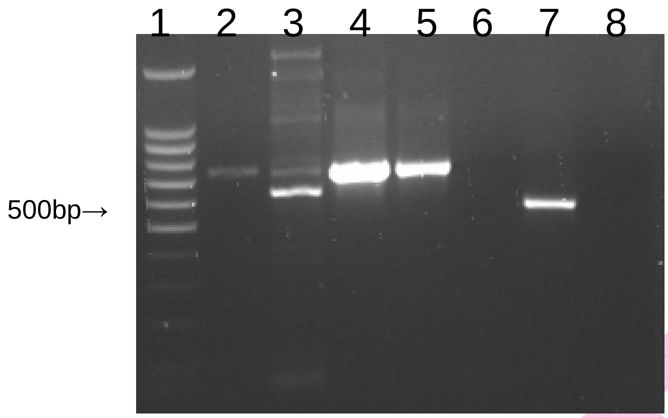

Figure 5.6.3. Amplification of the trypanosome 18S rRNA gene from woylie samples collected in the Upper Warren area. Lane 1: Molecular marker 100bp; Lane 2-6: woylie samples; Lane 7: positive control; Lane 8: negative control.

5.6.4. Discussion

Trypanosome prevalence, at 43% positive by light microscopy, appears relatively high within the Upper Warren woylie population, with further investigations using PCR based methods likely to reveal even higher levels (Desquesnes and Davila, 2002; Solano *et al.,* 1999). With little baseline data to refer to it is not possible to say whether this prevalence level is within a normal range or not, but it is in stark contrast to the zero prevalence level reported from within the non-declining Karakamia population.

There are several additional factors that must be taken into account when considering the potential role and effect of trypanosomes in the woylie decline. One of the most important is the prevalence and diversity of concomitant infections within the population. Within the Karakamia population, PCR-based methods have indicated that, contrary to light microscopy, there are some individuals positive for trypanosome infection. However, as reported in section 5.7, all samples screened so far (n = 81) for *Toxoplasma* using the modified agglutination test have been sero-negative, whereas the minimum prevalence within the Upper Warren population during March 2006 was 5.8% (Section 5.7 *Toxoplasma)*. As mentioned above, the effects of *Toxoplasma* have been shown to be more severe (increased tachyzoites count) when the host was also co-infected with trypanosomes. These synergistic effects are not clearly understood and, furthermore, it is not known whether such mixed infections adversely affect the impact or pathogenicity of trypanosomes.

There is also evidence to suggest that, even when considered as a singular infection, trypanosomes can have a direct negative impact on host fitness (Brown *et al.,* 2000). For example, Ratti *et al.* (1993) showed that male pied flycatchers (*Ficedula hypoleuca*) that were infected with a *Trypanosoma* species had poorer feather development and arrived at their destination several days later than uninfected males following their annual migration. If trypanosome infected woylies were to suffer a slight reduction in fitness, perhaps in a way that resulted in their becoming more prone to predation or the effects of other diseases in the environment, then that would amount to a significant effect at the population level considering the high overall prevalence of trypanosome infection reported above.

Aside from differences in prevalence levels and concomitant infections, there remains the possibility that a novel trypanosome species has been introduced into the region. This remains highly speculative at present until genotyping and sequencing results become available. However, initial sequencing results have indicated little difference between trypanosome species from either population which further suggests an additive effect of concomitant infections or environmental stress.

5.6.5. References

Brown, M, J. F., Loosli, R. and Schmid-Hempel, P. (2000) Condition-dependent expression of virulence in a trypanosome infecting bumblebees. Oikos 91: 421-427.

Cox, F. E. G. (2001) Concomitant infections, parasites and immune response. Parasitology 122: S23-S38.

Desquesnes, M. and Davila, A. M. R. (2002) Applications of PCR-based tools for the detection and identification of animal trypanosomes: a review and perspectives. Veterinary Parasitology 109: 213-231.

Guerrero, O. M., Chinchilla, M. and Abrahams, E. (1997) Increasing of *Toxoplasma gondii* infections by Trypanosoma lewisi in white rats. Revista De Biologia Tropical 45 (2): 877-882.

Hamilton, P.B., Stevens, J. R., Gidley, J., Holz, P. and Gibson, W. C. (2005). A new lineage of trypanosomes from Australian vertebrates and terrestrial bloodsucking leeches (Haemadipsidae). International Journal for Parasitology. 35 (4): 431-443.

Laakkonen, J. (2002) Trypanosome parasites in the invading Rattus rattus and endemic rodents in Madagascar. In Rats, Mice and People: Rodent Biology and Management (eds Singleton, G., Hinds, L., Krebs, C. and Spratt, M.) ACARI Canberra.

Laakkonen, J., Goodman, S. M., Duchemin, J. B. and Duplantier, J. M. (2003) Trypomastigotes and potential flea vectors of the endemic rodents and the introduced *Rattus rattus* in the rainforests of Madagascar. Biodiversity and Conservation 12: 1775-1783.

Maraghi, S. and Molyneux, D. H. (1989) Studies on cross-immunity in Herpetosoma trypanosomes of Microtus, Clethryonomus and Apodemus. Parasitology Research. 75 (3): 175-177.

Pickering, J. and Norris, C. A. (1996) new evidence concerning the extinction of the endemic murid *Rattus macleari* from Christmas Island, Indian Ocean. Australian Mammalogy 19: 1.

Ratti, O., Dufva, R. and Alatalo, R. V. (1993) Blood parasites and male fitness in the pied flycatcher. Oecologia 96 (3): 410-414.

Smith, A., Telfer, S., Burthe, S., Bennett, M. and Begon, M. (2005) Trypanosomes, fleas and field voles: ecological dynamics of a host-vector-parasite interaction. Parasitology 131: 355-365.

Solano, P., Michel, J. F., Lefrancois, T., De La Rocque, S., Sidibe, I., Zoungrana, A. and Cuisance, D. (1999) Polymerase chain reaction as a diagnosis tool for detecting trypanosomes in naturally infected cattle in Burkina Faso. Veterinary Parasitology 86: 95-103.

5.7. *Toxoplasma*

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Abstract

In response to the dramatic declines in woylie numbers, a number of woylies in the Upper Warren and in control populations outside of the Upper Warren were tested for *Toxoplasma* infection. The commercially available modified agglutination test (MAT) was used to detect *Toxoplasma* antibodies in woylie sera. A number of variables were analyzed to determine if *Toxoplasma* infection is contributing to the decline in woylie numbers in the Upper Warren. The retrap rates of *Toxoplasma* seropositive woylies was observed to determine if *Toxoplasma* seropositive woylies survive. In addition, the *Toxoplasma* seroprevalence of woylies in the Upper Warren was compared with woylies located elsewhere, where populations were not declining. In March 2006, 153 woylies from the Upper Warren were tested for *Toxoplasma* antibodies, nine were seropositive for *Toxoplasma*. In July to December of the same year, 143 more woylies from the Upper Warren were tested for *Toxoplasma*, zero seropositive. Although six out of the nine seropositive woylies were retrapped none of these woylies were re-bled in July to December 2006. All sera samples from outside of the Upper Warren, including Karakamia and Dryandra, showed a zero seroprevalence of *Toxoplasma* in woylies. Data to date suggests *Toxoplasma* might contribute to woylie deaths, however this is not conclusive. Future studies include continued monitoring of woylie populations for *Toxoplasma* antibodies and further analysis of woylie tissues for *Toxoplasma* DNA.

5.7.1. Introduction

Toxoplasma is a protozoan parasite that infects virtually all warm-blooded species, including humans and marsupials (Tenter, Heckeroth *et al.,* 2000). Infection with *Toxoplasma* can affect marsupials in several ways. Initial infection can result in acute Toxoplasmosis causing overt disease which often leads to death (Johnson, Roberts *et al.,*. 1989). Other symptoms of acute disease include lethargy, inappetence, respiratory distress and neurological disturbances. Alternatively, *Toxoplasma* can remain dormant in tissue resulting in long-term latent infection which can be reactivated during times of stress (Beveridge, 1993). Stressors which may cause reactivated Toxoplasmosis in marsupials include nutritional stresses, capture stress and transport and captivity stress (Obendorf and Munday, 1983; Beveridge, 1993). In addition, infection with *Toxoplasma* may make a marsupial more prone to predation by affecting its behaviour, movement, coordination and sight.

Felids are the only host of *Toxoplasma* that are capable of shedding oocysts (i.e. cats are the the definitive/final host). Cats shed large numbers of infective oocysts briefly during acute infection, after which it is rare for shedding to occur again. Oocysts can remain infective in soil for up to 2 years under favourable climatic conditions (Frenkel, Ruiz *et al.,*. 1975). Oocyst remain viable for longer periods of time in a warm and moist environment (Yilmaz and Hopkins, 1972). Herbivorous marsupials, may become infected with *Toxoplasma* through ingesting material contaminated with oocysts or through vertical transmission of tachyzoites. Evidence for vertical transmission in marsupials to date is anecdotal (Boorman, Kollias *et al.,* 1977; Dubey, Ott-Joslin *et al.,* 1988), however it is well established in a number of species including sheep, mice, rats, cats and humans (Johnson, 1997; Duncanson, Terry *et al.,* 2001; Marshall, Hughes *et al.,* 2004). Meat eating marsupials may also be infected via ingesting animal tissue containing *Toxoplasma bradyzoites* (cysts).

Several species of marsupial have been found to be infected with *Toxoplasma* using methods such as serology, histology and polymerase chain reaction (PCR) to detect *Toxoplasma* antibodies, organisms and DNA respectively. Outbreaks of acute toxoplasmosis causing widespread mortalities have been well reported in captive marsupials such as the long-nosed potoroo (*Potorous tridactylus*), tammar wallaby (*Macropus eugenii*), eastern grey kangaroo (*Macropus giganteus*), red kangaroo (*Macropus rufus*), wallaroo (*Macropus robustus*) and Bennett's wallaby (*Macropus rufogriseus*) *(Boorman, Kollias et al.,* 1977; Patton, Johnson *et al*., 1986; Basso, Venturini *et al.,* 2006). The impact of *Toxoplasma* infection in wild marsupials is more difficult to determine as predation of recently infected marsupials hinders investigation into the cause of death. *Toxoplasma* antibodies have been detected in several wild marsupial populations including the koomal (*Trichosurus vulpecula*) (Eymann, Herbert *et al*., 2006), Tasmanian pademelon (*Thylogale billardierii*) and Bennett's wallaby (Johnson, Roberts *et al.,* 1988). In-depth investigations regarding the impact of *Toxoplasma* in eastern-barred bandicoots (*Perameles gunnii*) led to the conclusion that *Toxoplasma* infection is a significant cause of death among this species, both in captivity and in the wild (Obendorf, Statham *et al.,* 1996; Bettiol, Obendorf *et al.,* 2000; Miller, Mitchel *et al.,* 2000). Based on this evidence it is clear that *Toxoplasma* is an infectious agent that needs to be included in the differential diagnosis list of investigations regarding the cause of decline in wild marsupial populations.

Initial investigations into the presence of *Toxoplasma* infection in woylie populations experiencing decline began using serology. Serology detects antibodies which are often widely dispersed in the blood stream and therefore easy to detect during blood screening. However, some animals, particularly marsupials, which are known to be highly susceptible to *Toxoplasma*-related disease, may not survive initial infection and die of acute toxoplasmosis before IgG antibodies can be detected. This is one of the limitations of serology in ascertaining the impact of *Toxoplasma* infection. In addition, serology alone is unable to verify if *Toxoplasma* is causing disease in infected animals. Additional data such as retrap rates of seropositive and seronegative woylies in addition to *Toxoplasma* serology from control groups needs to be analysed to determine the impact of *Toxoplasma* infection in a population. *Toxoplasma* detection using PCR and histology detect *Toxoplasma* present within animal tissue, necessitating invasive sampling techniques or necropsy. Furthermore, during chronic infection, *Toxoplasma* is spread sparsely within tissue and is consequently difficult to detect with PCR and histology. However, histology has the benefit of examining animal tissue infected with Toxoplasma therefore determining if *Toxoplasma* contributes to disease. The Woylie Conservation Research Project (WCRP) Operations Handbook (Volume 3) established (through the guidance of the Woylie Disease Reference Council; WDRC) that serum be tested from live animals whereas tissue from recently dead woylies was to be analyzed as part of routine histology and tissue samples set aside for *Toxoplasma* PCR.

The modified agglutination test (MAT) is a popular and reliable test for the serodiagnosis of *Toxoplasma* in Australian marsupials (Dubey, Ott-Joslin *et al.,* 1988; Miller, Faulkner *et al.,* 2003). It has been used as a sensitive and specific test in humans (Desmonts and Remington 1980) and a variety of animals such as mice (Dubey, Thulliez *et al.,* 1995), pigs (Dubey, Thulliez *et al.,* 1995), sheep (Ljungstrom, Lunden *et al.,* 1994) and felids (Dubey, Lappin *et al.,* 1995; Dubey, Navarro *et al.,* 2004). It is one of the few serological tests which can be used on a range of species, and therefore can be applied to woylies without the need for time-consuming and costly optimization and validation studies. The MAT was found to be one of the most sensitive tests to detect *Toxoplasma* antibodies in kangaroo sera, when compared to the dye test, indirect agglutination test and latex agglutination test (Dubey, Ott-Joslin *et al.,* 1988).

5.7.2. Methods

In March 2006, 153 woylies from the Upper Warren were bled from the lateral tail vein as part of DEC trapping and sampling programs for the WCRP. This initial sera sample set was expanded when a further 143 woylies from the Upper Warren were bled from July to December 2006. In addition, sera from woylie populations elsewhere were also obtained. These sera samples were obtained from other declining or declined woylie populations at Dryandra (n=12), Batalling (n=17) and Venus Bay (n=14), and stable populations at Karakamia (n=81), Tutanning (n=8), St Peter Island (n=72).

Sera was tested using the commercially available MAT kit (Toxo-Screen DA, bioMerieux, REF 75 481) at two different sera dilutions; 1:40 and 1:4000 and the test undertaken according to the manufacturer's directions. The positive and negative control sera included in the kit were used in each round of samples tested, in addition to a PBS control. A sera sample was determined to be *Toxoplasma* positive when an agglutination reaction was observed at a dilution of at least 1:40, based on the manufacturer's directions.

During the *Toxoplasma* seroprevalence study of woylies, opportunistic sampling of dead or euthanized woylies for the detection of *Toxoplasma* was also undertaken. Any woylies that were submitted for routine necropsy were examined by the Duty Pathologist at Murdoch University, School of Veterinary and Biomedical Sciences, and had tissue samples removed for histology, in which *Toxoplasma* infection was screened for as a differential diagnosis. In addition, tissues including the brain, heart and skeletal muscle were set aside from each woylie necropsied to test for *Toxoplasma* DNA using PCR (Bretagne, Costa *et al.,* 1993). Samples of heart blood were also taken in necropsied woylies in which blood was deemed fresh. Sera was then obtained from this blood to test for *Toxoplasma* antibodies. In addition, some woylie pouch-young (injured, predated or orphaned) were dissected or frozen for *Toxoplasma* DNA screening.

5.7.3. Results

Toxoplasma antibodies were only detected in nine (5.8%) woylies sampled in the Upper Warren in March 2006. None of 143 woylies sampled from the Upper Warren in July-December 2006 were seropositive. The change in seroprevalence in the Upper Warren is significantly different (Fisher's exact test; p=0.0036). All other sera samples tested were classified as *Toxoplasma* seronegative and had MAT titers of <1:40 (Table 5.8.1). Assuming that the serology tests do not produce false negatives, the sample size from Karakamia (n=81) is sufficient to have 95% confidence that the actual prevalence of *Toxoplasma* within the population is 0-5%, based on point and interval estimates (Snedecor, 1956). The samples sizes at Dryandra, Tutanning and Batalling are not sufficient to confidently determine whether or not *Toxoplasma* may be present at low prevalences. For example, if the actual prevalence in a population is 5%, more than 59 samples are required to have a probability of less than 5% that all the samples are negative (SAS).

Six out of the nine *Toxoplasma* seropositive woylies were found to have been retrapped seven to 13 months after initial trapping in March 2006 (Table 5.7.2). Given that trapping intensity and frequency at the Upper Warren sites were sufficiently high, it is likely that the retrap rates have a high correlation with "loss" of those individuals from the trapped population, most likely through mortality. It is therefore probable that the three seropositive woylies that were not retrapped died. Further analysis needs to be undertaken to compare the retrap rates of seronegative and seropositive woylies, to ascertain if seropositive woylies have a higher mortality rate.

On 31 necropsies undertaken on woylies from various sites (including Karakamia, Dryandra, Tutanning and Upper Warren), there was no evidence of infection with *Toxoplasma* based on routine histology. A limited amount of tissue samples from necropsied woylies have been tested for *Toxoplasma* DNA using PCR, however out of the three tissue samples tested, no *Toxoplasma* DNA was detected. There are several tissue samples stored, from a further 28 woylies, and these samples will be tested for *Toxoplasma* DNA shortly. One out of five woylie pouch-young submitted has been tested for *Toxoplasma* DNA. *Toxoplasma* DNA was identified in a brain sample of this pouch-young. This result needs to be verified with DNA sequencing.

Table 5.7.1. *Toxoplasma* seroprevalence based on the MAT.

Location		Seropositive	Total tested
Upper Warren- March	Balban	4	36
Upper Warren- March	Boyicup	0	4
Upper Warren- March	Chariup	0	4
Upper Warren- March	Corbal	2	26
Upper Warren- March	Keninup	2	34
Upper Warren- March	Warrup	0	22
Upper Warren- March	Winnejup	0	5
Upper Warren- March	Yendicup	1	8
Upper Warren- March	Misc	0	14
Summary Upper Warren - March 06		9	153
Upper Warren- Jul-Dec	Balban	0	27
Upper Warren- Jul-Dec	Boyicup	0	6
Upper Warren- Jul-Dec	Keninup	0	59
Upper Warren- Jul-Dec	Warrup	0	39
Upper Warren- Jul-Dec	Winnejup	0	12
Summary Upper Warren - Jul-Dec 06		0	143
Karakamia - Jul'06		0	81
Dryandra - Nov 06		0	12
Tutanning - Nov 06		0	8
Batalling - Nov 06		0	17

Table 5.7.2. Retrap data of *Toxoplasma* seropositive woylies

Date	Forest Block	Animal Record No.	Sex	Weight g	Age
23/03/2006*	Balban	500003370	M	1410	A
24/3/2006	Balban	500003370	M		A
4/11/2004	Balban	90000815	F	1300	A
5/11/2004	Balban	90000815	F	1260	A
18/11/2004	Balban	90000815	F	1150	A
2/11/2005	Balban	90000815	F	1340	A
3/11/2005	Balban	90000815	F	1280	A
23/03/2006*	Balban	90000815	F	1400	A
23/03/2006*	Balban	500003363	M	1570	A
27/3/2007	Balban	500003363	M	1520	A
30/3/2007	Balban	500003363	M	1540	A
17/11/2004	Balban	500003200	M	1300	A
23/03/2006*	Balban	500003200	M	1500	A
29/3/2007	Balban	500003200	M	1570	A
8/12/2005	Corbal	500002496	M	1460	A
16/03/2006*	Corbal	500002496	M	1380	A
17/3/2006	Corbal	500002496	M	1370	A
23/11/2006	Corbal	500002496	M	1550	A
17/4/2007	Corbal	500002496	M	1500	A
16/03/2006*	Corbal	500003323	M	1480	A
22/11/2006	Corbal	500003323	M	1650	A
18/4/2007	Corbal	500003323	M	1650	A
20/4/2007	Corbal	500003323	M	1700	A
28/03/2006*	Keninup	500003058	M	1250	A
31/3/2006	Keninup	500003058	M	1250	A
27/3/2007	Keninup	500003058	M	1275	A
28/3/2007	Keninup	500003058	M	1200	A
30/3/2007	Keninup	500003058	M	1225	A
9/11/2005	Keninup	500002560	M	1450	A
29/03/2006*	Keninup	500002560	M	1475	A
31/10/2006	Keninup	500002560	M	1375	A
4/04/2006*	Yendicup	500004276	M	1550	A

("*" denotes the date in which woylie sera was sampled for *Toxoplasma* antibodies, "A" under the column heading of Age, refers to "adult")

5.7.4. Discussion

The change in the Upper Warren region in the seroprevalence of *Toxoplasma* from 5.8% (n=153) to 0% (n=143) between March and July-December 2006 is statistically highly significant (Fisher's exact test; p=0.0036). The significant difference in *Toxoplasma* seroprevalence values among the same population of woylies at different time periods is epidemiologically consistent with a disease agent causing population decline. *Toxoplasma* infection in marsupials can result in acute disease and rapid death (Canfield, Hartley *et al.,* 1990). Acute disease can ensue soon after initial *Toxoplasma* infection, or after a period of chronic infection (Beveridge, 1993). Reactivated toxoplasmosis after chronic *Toxoplasma* infection has been found to occur after a "stressor" in some marsupial case studies. Therefore, there are two possible causes of widespread Toxoplasmosis in woylies in the Upper Warren; a recent increase of felids which are shedding oocysts or a recent "stressor" causing reactivated Toxoplasmosis of chronically infected woylies.

Felids have been found to shed oocysts in their faeces for only one to two weeks after initial infection with *Toxoplasma* (Dubey and Frenkel, 1972; Dubey and Frenkel, 1976). Although it is very rare for cats to reshed oocysts later in life, this has been reported in cats infected with other benign coccidian parasites, during immunosuppression or after re-exposure to *Toxoplasma* years after initial infection (Dubey and Frenkel, 1974; Dubey, 1976; Dubey, 1995). Cats can obtain *Toxoplasma* from ingesting infected meat, ingesting oocyst contaminated feed or via congenital transmission. As most cats only shed once in their life, kittens and juvenile cats are often the source of *Toxoplasma* oocysts, as opposed to mature cats.

One hypothesis to explain an increased exposure of woylie populations to *Toxoplasma* is that an increased number of oocysts have been introduced to the environment. This could occur if one or more cats have ingested *Toxoplasma* infected meat. In addition, the total number of cats shedding oocysts in any given location can increase dramatically when acutely infected cats transmit *Toxoplasma* to their kittens. Therefore, there is potential for many cats to become acutely infected and consequently shed oocysts over a short period of time. Domestic cats bury their faeces in soil. Because woylies dig soil to obtain feed, this places them at a higher risk of ingesting oocyst contaminated feed compared to non-burrowing animals. An additional hypothesis as to how *Toxoplasma* can be introduced to a woylie population is that non-felid *Toxoplasma* infected species have been introduced which woylies have ingested via opportunistic scavenging.

Subclinical, chronic *Toxoplasma* infection in marsupials can become acute disease by stressors such as capture, transportation and captivity, malnourishment and extreme weather (Arundel, Barker *et al.,* 1977; Obendorf and Munday, 1983; Obendorf and Munday, 1990). Reactivated Toxoplasmosis is also commonly reported in immunosuppressed humans such as AIDS patients (Boothroyd and Grigg, 2002) and organ transplant patients (Wendum, Carbonell *et al.,* 2002). A secondary stressor has the potential to cause a reactivated, fatal Toxoplasmosis in subclinically infected woylies. A hypothesis to explain an increased level of fatal Toxoplasmosis in woylies is that *Toxoplasma* was introduced to the woylie population years prior to a secondary stressor that then caused reactivated, acute disease. Initial *Toxoplasma* infection results from ingesting infected meat or oocyst-contaminated feed and congenital infection. Given that *Toxoplasma* DNA was identified in one woylie pouch-young, this suggests that congenital transmission of *Toxoplasma* does occur in woylies. However, this result needs to be confirmed by additional tests.

The trapping records of the nine seropositive woylies captured in March 2006 showed that six were recaptured, 7 to 13 months later (Table 5.7.2). This demonstrates that some individuals can survive with *Toxoplasma*, at least in the short term. Two past seropositive woylies were rebled in 2007 trapping regimes, and these sera will soon be tested for *Toxoplasma* antibodies in order to investigate changes in immune status.

There were no seropositive results for *Toxoplasma* in woylies from all samples outside of the Upper Warren. These included populations that were not experiencing decline (i.e. Karakamia and Tutanning), and some very limited samples from populations that have declined (Dryandra, Batalling). The absence of detected *Toxoplasma* in stable woylie populations provides some (weak) comparative evidence that may implicate Toxoplasmosis as having a role in recent woylie declines. By default it also remains unknown if *Toxoplasma* can remain in a woylie population without causing (or contributing to) a dramatic decline. The strength of the evidence is greatly limited by the sample sizes collected to date, relative to the sample sizes needed to confidently assess the presence/absence of an agent that may occur at low prevalence levels. Other populations of woylies (St Peter's Island and Venus Bay Island A) not experiencing decline have been bled to test for *Toxoplasma* antibodies, however, they remain to be analysed

Toxoplasma antibodies have been detected in other species of marsupial in the wild, including the koomal (Eymann, Herbert *et al.,* 2006), eastern-barred bandicoots (Obendorf, Statham *et al.,* 1996), Tasmanian pademelons and Bennett's wallabies (Johnson, Roberts *et al.,* 1988). Of these species, *Toxoplasma* has been confirmed as a contributor to deaths in the eastern-barred bandicoots and speculated to have caused deaths in the koomal. In addition, a case report of Toxoplasmosis in wild Tasmanian pademelons, where two carcasses were examined histologically, found *Toxoplasma* to be the cause of death (Obendorf and Munday, 1983). These two wallabies were found stumbling blindly and were subsequently euthanized. According to the land-owner where the wallabies were found, sick and dead wallabies had been observed every year, with the number of wallabies affected increasing yearly (Obendorf and Munday, 1983). From this case study it appears that *Toxoplasma* did contribute to Tasmanian pademelon deaths, however the overall population of Tasmanian pademelons in Tasmania remained relatively stable (Hocking, 2007).

Eastern-barred bandicoots have been found to be highly susceptible to death when infected with *Toxoplasma* in the wild (Obendorf, Statham *et al.,* 1996). In a study of free ranging eastern-barred bandicoots, a seroprevalence of 6.3% was found in 133 animals tested. Of the 10 seropositive animals, five were not retrapped, and of the remaining bandicoots, one was found dead in the trap with generalised toxoplasmosis (diagnosed at necropsy) while another had central nervous system disabilities consistent with Toxoplasmosis but was accidentally released and never recaptured (Obendorf, Statham *et al.,* 1996). During the period of this study, eight dead eastern-barred bandicoots were examined by necropsy and seven were confirmed as having toxoplasmosis. Based on the combination of findings, it was concluded that eastern-barred bandicoots are likely to be highly susceptible to primary *Toxoplasma* infection.

A published study of the koomal found a *Toxoplasma* seroprevalence of 6.3% in 142 animals tested. *Toxoplasma* was implicated as a contributor to deaths in these koomals based on the retrap success of the seropositive koomals. Only one out of nine seropositive animals was recaptured, however recapture success for seronegative possums declined over the sampling period from 57-35%. This reduction in koomal retrap rates may have also been influenced by other factors such as exposure to other diseases, road kill, illegal relocation and/or trap shyness (Eymann, Herbert *et al.,* 2006).

Further data analysis needs to be undertaken to compare the retrap rates of seronegative and seropositive woylies to ascertain if seropositive woylies that were not retrapped may have died from *Toxoplasma*. The ideal way to determine if *Toxoplasma* is killing woylies is to find dead or diseased woylies with signs of *Toxoplasma* infection, such as what occurred in the eastern-barred bandicoot study (Obendorf, Statham *et al.,* 1996). Toxoplasmosis has caused rapid and substantial declines in the wild in Californian sea otters (Miller *et al*., 2004) and less dramatic declines in eastern barred bandicoots (Obendorf *et al*., 1996). Diagnosis of *Toxoplasma* being the cause of decline in these species was strongly aided by the examination of dead animals via histology and, in the case of the otters, *Toxoplasma* PCR. However, a major problem that inhibits obtaining dead or dying woylies is the predation of infected woylies. In addition, when dead woylies are obtained they are often not in fresh enough condition to obtain a thorough analysis of Toxoplasmosis lesions. Although Toxoplasmosis related lesions are often evident in animals that have died peracutely as a result of the disease, identifying these lesions is difficult in carcasses that are frozen or not fresh (Canfield *et al*., 1990).The experimental infection of woylies with *Toxoplasma* in order to study the pathological effects directly is also problematic. The response of marsupials to *Toxoplasma* in captivity may bare no relation to their response in wild populations.

5.7.5. Conclusion

While there is not direct evidence, data to date is consistent with *Toxoplasma* potentially contributing to woylie deaths. Further studies, as outlined below, need to be undertaken to better identify the impact of *Toxoplasma* infection in wild woylies. The diagnosis of Toxoplasmosis in a dead or diseased woylie in the Upper Warren would add significantly to the evidence that *Toxoplasma* may be involved in the woylie declines.

5.7.6. Future work

Future studies include PCR analysis of stocks of woylie tissue from dead animals associated with the PCS Survival and mortality study (Section 4.3) and/or animals that have undergone necropsy. Sequencing of *Toxoplasma* DNA found in the brain of a woylie pouch-young needs to be undertaken to confirm the pouch-young was infected with *Toxoplasma*. Additional woylie sera samples collected from Venus Bay, St Peter’s Island and the Upper Warren (2007) need to be tested for *Toxoplasma* antibodies using the MAT. Data analysis needs to be undertaken to compare the retrap rates of *Toxoplasma* seropositive and seronegative woylies.

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5.7.8. References

Arundel, J. H., I. K. Barker, *et al.* (1977). Diseases of marsupials. The Biology of Marsupials. B. Stonehouse and D. Gilmore. London, Macmillan: 141-154.

Basso, W., M. C. Venturini, *et al.* (2006). "Toxoplasmosis in captive Bennett's wallabies (*Macropus rufogriseus*) in Argentina." Vet Parasitol.

Bettiol, S. S., D. L. Obendorf, *et al.* (2000). "Pathology of experimental toxoplasmosis in eastern barred bandicoots in Tasmania." J Wildl Dis 36(1): 141-4.

Beveridge, I. (1993). Marsupial parasitic diseases. Zoo and wild animal medicine : current therapy. M. Fowler. Philadelphia, W.B. Saunders: 288-293.

Boorman, G. A., G. V. Kollias, *et al.* (1977). "An outbreak of toxoplasmosis in wallaroos (*Macropus robustus*) in a California zoo." J Wildl Dis 13(1): 64-8.

Boothroyd, J. C. and M. E. Grigg (2002). "Population biology of *Toxoplasma gondii* and its relevance to human infection: do different strains cause different disease?" Curr Opin Microbiol 5(4): 438-42.

Bretagne, S., J. M. Costa, *et al.* (1993). "Detection of *Toxoplasma gondii* by competitive DNA amplification of bronchoalveolar lavage samples." J Infect Dis 168(6): 1585-8.

Canfield, P. J., W. J. Hartley, *et al.* (1990). "Lesions of toxoplasmosis in Australian marsupials." J Comp Pathol 103(2): 159-67.

Desmonts, G. and J. S. Remington (1980). "Direct agglutination test for diagnosis of *Toxoplasma* infection: method for increasing sensitivity and specificity." J Clin Microbiol 11(6): 562-8.

Dubey, J. P. (1976). "Reshedding of *Toxoplasma* oocysts by chronically infected cats." Nature 262(5565): 213-4.

Dubey, J. P. (1995). "Duration of immunity to shedding of *Toxoplasma gondii* oocysts by cats." J Parasitol 81(3): 410-5.

Dubey, J. P. and J. K. Frenkel (1972). "Cyst-induced toxoplasmosis in cats." J Protozool 19(1): 155-77.

Dubey, J. P. and J. K. Frenkel (1974). "Immunity to feline toxoplasmosis: modification by administration of corticosteroids." Vet Pathol 11(4): 350-79.

Dubey, J. P. and J. K. Frenkel (1976). "Feline toxoplasmosis from acutely infected mice and the development of *Toxoplasma* cysts." J Protozool 23(4): 537-46.

Dubey, J. P., M. R. Lappin, *et al.* (1995). "Diagnosis of induced toxoplasmosis in neonatal cats." J Am Vet Med Assoc 207(2): 179-85.

Dubey, J. P., I. T. Navarro, *et al.* (2004). "*Toxoplasma gondii* infections in cats from Parana, Brazil: seroprevalence, tissue distribution, and biologic and genetic characterization of isolates." J Parasitol 90(4): 721-6.

Dubey, J. P., J. Ott-Joslin, *et al.* (1988). "Toxoplasmosis in black-faced kangaroos (*Macropus fuliginosus melanops*)." Vet Parasitol 30(2): 97-105.

Dubey, J. P., P. Thulliez, *et al.* (1995). "*Toxoplasma gondii* in Iowa sows: comparison of antibody titers to isolation of *T. gondii* by bioassays in mice and cats." J Parasitol 81(1): 48-53.

Dubey, J. P., P. Thulliez, *et al.* (1995). "Sensitivity and specificity of various serologic tests for detection of *Toxoplasma gondii* infection in naturally infected sows." Am J Vet Res 56(8): 1030-6.

Duncanson, P., R. S. Terry, *et al.*. (2001). "High levels of congenital transmission of *Toxoplasma gondii* in a commercial sheep flock." Int J Parasitol 31(14): 1699-703.

Eymann, J., C. A. Herbert, *et al.* (2006). "Serologic survey for *Toxoplasma gondii* and Neospora caninum in the common brushtail possum (*Trichosurus vulpecula*) from urban Sydney, Australia." J Parasitol 92(2): 267-72.

Frenkel, J. K., A. Ruiz, *et al.* (1975). "Soil survival of *Toxoplasma* oocysts in Kansas and Costa Rica." Am J Trop Med Hyg 24(3): 439-43.

Hocking, G. (2007). Tasmanian Pademelon, Department of Primary Industries and Water.

Johnson, A. M. (1997). "Speculation on possible life cycles for the clonal lineages in the genus *Toxoplasma*." Parasitol Today 13(10): 393-7.

Johnson, A. M., H. Roberts, *et al.* (1988). "Prevalence of *Toxoplasma gondii* antibody in wild macropods." Aust Vet J 65(7): 199-201.

Johnson, A. M., H. Roberts, *et al.* (1989). "Serodiagnosis of acute toxoplasmosis in macropods." Vet Parasitol 34(1-2): 25-33.

Ljungstrom, B. L., A. Lunden, *et al.* (1994). "Evaluation of a direct agglutination test for detection of antibodies against *Toxoplasma gondii* in cat, pig and sheep sera." Acta Vet Scand 35(2): 213-6.

Marshall, P. A., J. M. Hughes, *et al.* (2004). "Detection of high levels of congenital transmission of *Toxoplasma gondii* in natural urban populations of *Mus domesticus*." Parasitology 128(Pt 1): 39-42.

Miller, D. S., C. Faulkner, *et al.* (2003). "Detection of *Toxoplasma gondii* IgG antibodies in juvenile great grey kangaroos, *Macropus giganteus giganteus*." J Zoo Wildl Med 34(2): 189-93.

Miller, D. S., G. F. Mitchel, *et al.* (2000). "Agglutinating antibodies to *Toxoplasma gondii* in sera from captive eastern barred bandicoots in Australia." J Wildl Dis 36(2): 213-8.

Obendorf, D. L. and B. L. Munday (1983). "Toxoplasmosis in wild Tasmanian wallabies." Aust Vet J 60(2): 62.

Obendorf, D. L. and B. L. Munday (1990). Toxoplasmosis in wild Eastern Barred Bandicoots, *Perameles gunnii*. Bandicoots and bilbies. C. M. Seebeck, P. R. Brown, R. L. Wallis and C. M. Kemper. Sydney, Australia, Surrey Beatty in association with Australian Mammal Society: 61-66.

Obendorf, D. L., P. Statham, *et al.*. (1996). "Detection of agglutinating antibodies to *Toxoplasma gondii* in sera from free-ranging eastern barred bandicoots (Perameles gunnii)." J Wildl Dis 32(4): 623-6.

Patton, S., S. L. Johnson, *et al.*. (1986). "Epizootic of toxoplasmosis in kangaroos, wallabies, and potaroos: possible transmission via domestic cats." J Am Vet Med Assoc 189(9): 1166-9.

Tenter, A. M., A. R. Heckeroth, *et al.*. (2000). "*Toxoplasma gondii*: from animals to humans." Int J Parasitol 30(12-13): 1217-58.

Wendum, D., N. Carbonell, *et al.* (2002). "Fatal disseminated toxoplasmosis in a *Toxoplasma* seropositive liver transplant recipient." J Clin Pathol 55(8): 637.

Yilmaz, S. M. and S. H. Hopkins (1972). "Effects of different conditions on duration of infectivity of *Toxoplasma gondii* oocysts." J Parasitol 58(5): 938-9.

5.8. Endoparasites

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Abstract

Very little research has been published regarding the prevalence and the effects of gastrointestinal parasites on native wildlife in southwestern Australia. Faecal samples from woylie populations in the Upper Warren and Karakamia were screened using microscopy for the presence of gastro-intestinal parasites. Information regarding general condition was also collected. In the Upper Warren, over 80% of all animals sampled over a ten year period were infected with at least one parasite species, and there was little difference over time in either parasite prevalence or parasite diversity. For faecal samples collected from the Upper Warren and Karakamia populations in 2006, prevalence of parasitic infection was significantly greater in Upper Warren (100%) than in Karakamia (92%), due principally to a greater prevalence of nematode larvae in the faecal samples. Preliminary data found no significant correlation between the presence of gastro-intestinal parasites and the general condition of the woylies, although the analyses were hampered by a lack of resolving power in discriminating among parasite species.

5.8.1. Introduction

There have been very few studies conducted focusing on gastro-intestinal parasitism amongst Australian wildlife in southwestern Australia. The only study of enteric parasites in native wildlife in southwestern Australia was by Adams (2003). In this study, a number of different species of native wildlife and feral cats were screened for gastro-intestinal parasites in southwestern Australia. A high prevalence of gastro-intestinal parasites was found in Australian native wildlife. Seventy two percent of animals were infected with at least one parasitic species. A number of different parasitic species were detected in these animals. The most common nematode species found were strongyle nematodes and *Strongyloides*. Many protozoan species were also detected, including *Entamoeba*, *Giardia* and unidentified coccidia species (Adams, 2003). Recently, *Blastocystis* was also detected in koomals (Parkar *et al.,* 2007).

The Woylie Conservation Research Project (WCRP) and the Australian Research Council (ARC) linkage project with DEC, provided an excellent opportunity to conduct a thorough study focusing on gastrointestinal parasites in various native species and populations in Western Australia. It would also shed light on whether infectious disease has any impact on the decline of the woylie population in Western Australia, and if so, what is the impact of gastro-intestinal parasitism and parasitic co-infections on these animals.

5.8.2. Methods

5.8.2.1. Samples

Two sets of data were available:

(1) A historical data set, consisting of records from faecal analyses of woylies from the Upper Warren, collected in 1998 (n = 63), 1999 (n=45), 2005 (n=11) and 2006 (n=51).

(2) A contemporary data set, consisting of 51 faecal samples collected from woylies in the Upper Warren in March 2006, and 60 faecal collected from woylies in Karakamia in July 2006.

5.8.2.2. Faecal analysis

All samples were fixed in 10% formalin for microscopy analysis, and 70% ethanol for analysis using molecular tools. For microscopy, all samples were concentrated using zinc-flotation and examined using light microscopy at a magnification of x100 for the presence of parasitic ova, larvae or trophozoites. Parasites were identified to the lowest possible taxonomic category.

5.8.2.3. Data analysis

Each individual woylie sample was recorded as being positive or negative for any parasitic infection, and for infection with each taxonomic category of parasites. Populations of woylies were compared for prevalence (percentage of infected hosts) and for diversity of parasite taxa, as measured by Margalef's index (d) or the Shannon-Weiner diversity index (H). For the contemporary samples from the Upper Warren and Karakamia we obtained information on sex, age (adult or subadult), weight and pes length for each woylie from the DEC database. Condition score for each woylie was calculated as a residual from the regression of log weight on log long pes length.

Differences in parameter estimates among groups were compared by Chi-square or Fisher exact tests for categorical data and by analysis of variance for continuous data. All data were checked for normality and transformed, if necessary, prior to analysis. Where multiple tests of the same hypothesis were made, a Bonferroni correction was used to obtain an experiment-wide error rate of 0.05.

5.8.3. Results

5.8.3.1. Historical data

There was a relatively stable endoparasite fauna in woylies sampled from the Upper Warren between 1996 and 2006. Overall prevalence of parasitic infection (i.e. infection with any parasite taxon) was greater than 80% in all years (Figure 5.8.1(a)) and there was little change in parasite taxon diversity over time (Figure 5.8.1(b)).

The most common parasites over all years were eggs of strongyle nematodes, with nematode larvae also seen relatively frequently, especially in 2006 (Figure 5.8.2).

Figure 5.8.1(a)

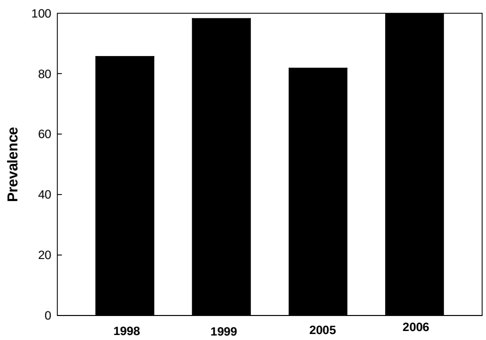

Figure 5.8.1(b)

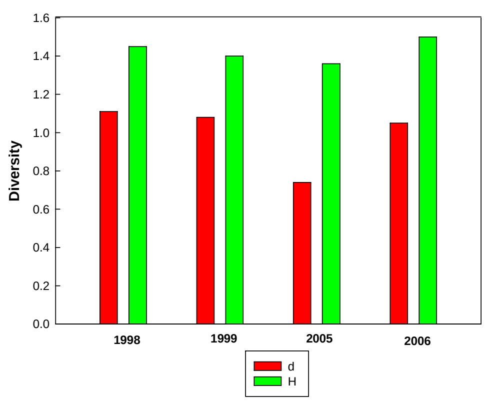

Figure 5.8.1. Prevalence of parasitic infection (a) and parasite taxon diversity in woylies from the Upper Warren over a ten year period.

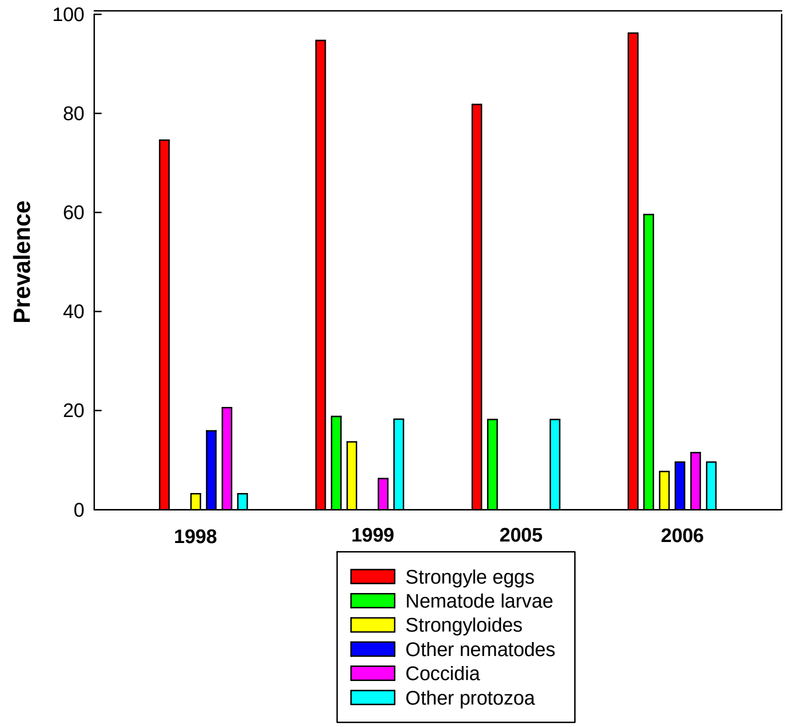

Figure 5.8.2. Prevalence of different parasite taxa in woylies from the Upper Warren over a ten year period.

5.8.3.2. Contemporary data

Ninety six percent of all woylies sample from the Upper Warren and Karakamia in 2006 were infected with at least one parasite taxon. In total, nine different taxa of gastrointestinal parasites were detected:

1. Strongyle eggs
2. Strongyloides eggs
3. Nematode larvae
4. Lungworm larvae
5. Echinonema eggs
6. Anoplocephalid eggs
7. Arthropod eggs
8. Coccidia spp
9. Entamoeba sp

Strongyle eggs were again the most prevalent taxon of parasites, with 92% of all woylies infected. There were no significant effects of either sex or age of woylies on the prevalence of any parasite taxon.

All woylies from Upper Warren were infected with at least one parasite taxon, compared with 92% from Karakamia (significant difference in prevalence, Fisher exact test, $P < 0.05$). When each parasite taxon was compared separately, both nematode larvae (60% prevalence in Upper Warren, 28% in Karakamia; Fisher exact test, $P < 0.05$ with the Bonferroni correction) and arthropod eggs (23% prevalence in Upper Warren, 0% in Karakamia; Fisher exact test, $P < 0.05$ with the Bonferroni correction) were significantly more prevalent in the Upper Warren population.

Although woylies from Upper Warren were in better condition (0.03 ± 0.02) than woylies from Karakamia (-0.03 ± 0.02), this was most likely due to the significantly smaller weights of Karakamia woylies, relative to size – a likely response to limited food resources and higher fauna densities at Karakamia (Section 4.2 Demographics). A two factor analysis of variance showed that condition was significantly affected by age (adults 0.02 ± 0.01, subadults -0.11 ± 0.04; $F = 4.31$, $P < 0.05$), but not by location or the interaction of age and location.

There were no significant effects of parasite infection (either overall or for each parasite taxon separately) on the condition of woylies from either Upper Warren or Karakamia.

5.8.4. Discussion

As expected, most woylies are infected with gastrointestinal parasites, particularly with strongyle nematodes, although a range of other nematode, cestode and protozoan parasite taxa were also found. Both the prevalence of parasite infection and the composition of the parasite fauna in the Upper Warren woylie population have changed little over the last ten years.

Woylies from the Upper Warren are infected more frequently with gastrointestinal parasites than are woylies from Karakamia, but there is no evidence that parasitic infection is associated with the condition of animals in either location. At this stage, the role of gastrointestinal parasites in woylie declines is undetermined. Some of the parasites which have been found, particularly strongyle nematodes and coccodian protozoa, have the potential to produce severe parasitic disease. Theoretical and empirical studies in other systems have shown that parasitic disease can be an important regulator of host population dynamics, at the prevalence levels which were found in this study (Cornell, 2006; Hudson *et al.,* 1998). The greater prevalence of some parasites in the Upper Warren than in Karakamia is suggestive of a regulatory role. On the other hand, there is no indication that parasitic infection reduces woylie condition, at least as measured by weight and pes length. It is possible that the effects of parasitic infection are more subtle, acting at a physiological or behavioural level that increases the susceptibility of the hosts to other mortality factors, such as predation or competition. Such indirect effects of parasitism have been reported in a number of other species of free living animals (Marcogliese, 2004)

5.8.5. Future work

One major limitation of this study has been the inability to distinguish parasite species using microscopy. This necessitated the use of higher taxon levels for analysis, which has undoubtedly reduced our ability to resolve parasitic effects upon the animals. In particular, there are almost certainly a number of different strongyle nematode species, as well as a number of unidentified coccidia in the faecal samples, undoubtedly representing new species. In order to identify these organisms, molecular tools must be used. However, considering the large number of samples to be screened, a multiplex technique is necessary in order to screen these samples more efficiently. An example of such a method is pyrosequencing, as it has both a built-in quality control and allows for the detection of mixed genotypes. It is also convenient, as there is no need for post PCR-purification, (Unemo *et al.,* 2004). The development of a pyrosequencing method is currently underway as part of Unaiza Parkar's PhD research.

After thoroughly screening the samples using molecular tools, further correlations and statistical analyses may be made based on co-infections of gastro-intestinal parasites identified to the species level, clinical signs and general health. A large number of faecal samples remain available for further investigation pending the development of results from the existing subsamples that have been analysed. These will also be made more generally available for more detailed investigations by future researchers or students.

5.8.6. Conclusion

Gastrointestinal parasite infections were more prevalent in Upper Warren than in the Karakamia woylie population, but preliminary data analysis has found no evidence of a relationship between infection and woylie condition. Nevertheless, it is possible that the differences in parasitism between populations may have important indirect effects on host mortality, which should be further investigated.

5.8.7. Acknowledgements

We would like to thank the DEC and AWC field staff and volunteers that assisted in the collection of faecal samples from the field and the associated data management.

5.8.8. References

Adams, P. J. 2003. Parasites of Feral Cats and Native Fauna from Western Australia: The Application of Molecular Techniques for the Study of Parasitic Infections in Australian Wildlife. Murdoch University, 1-239

Cornell, S. 2006. Modelling nematode populations: twenty years of progress. Trends in Parasitology **21**: 542-545.

Hudson, P.J., Dobson, A.P. and Newborn, D. 1998. Prevention of population cycles by parasite removal. Science **282**: 2256-2258.

Marcogliese, D.J. 2004. Parasites: small players with crucial roles in the ecological theatre. EcoHealth **1**: 151-164.

Parkar, U., Traub, R. J., Kumar, S., Mungthin, M., Vitali, S., Leelayoova, S., Morris, K. and Thompson, R. C. A. 2007. Direct characterization of Blastocystis from faeces by PCR and evidence of zoonotic potential. Parasitology **134:** 359-67

Unemo, M., Olcen, P., Jonasson, J. and Fredlund, H. 2004. Molecular Typing of *Neisseria gonorrhoeae* Isolates by Pyrosequencing of Highly Polymorphic Segments of the *porB* Gene. Journal of Clinical Microbiology **42:** 2926-2934

5.9. Ectoparasites

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Abstract

Although the importance of disease in the regulation of wildlife populations is well recognized and outbreaks of disease have been implicated in the decline or extinction of endangered species (Viggers *et al.*, 1993), there is a paucity of data about the prevalence and impact of pathogens and parasites on wildlife. This paper reports the preliminary findings regarding the ectoparasites of the woylie and sympatric species. New host records for two fleas are noted. A tick found on a number of woylies and one koomal appears to be a new species of *Ixodes.* Further work planned will examine host-parasite dynamics and some of the potentially pathogenic organisms carried by the ectoparasites such as trypanosomes and *Rickettsia*.

5.9.1. Introduction

Ectoparasites can have a variety of detrimental effects on host survival or fecundity through such diverse mechanisms as blood and energy loss (Khokhlova *et al.*, 2002), indirect changes to aspects of behaviour such as territoriality and territory size (Hoodless *et al.*, 2002; Whiteman and Parker, 2004) and the transmission of pathogens such as trypanosomes (Hamilton et. al., 2005, Laaakonen *et al.*, 2002) and *Rickettsia*. This project aims to describe the biodiversity of the ectoparasites of woylies and sympatric species, some features of the host-parasite relationship and investigate some of the pathogens they act as vectors for. This research is done within the context of understanding what role ectoparasites might play in the recent woylie declines.

5.9.2. Methods

Ectoparasites were collected from woylies and other mammals trapped in Western Australia as part of the Woylie Conservation Research Project. The sampling regime covered different seasons and habitat types. Ectoparasite burden was recorded by type (fleas, ticks, lice, mites). Small numbers of parasites were counted exactly and numbers of more abundant ectoparasites recorded semi-quantitatively using an ordinal scale. Ectoparasites were collected directly from animals into plain vials, which were transported chilled to Murdoch for live processing before fixing in 70% alcohol. Handling bags were shaken out between animals to reduce the possibility of mobile ectoparasites moving between animals. In later trapping sessions the technique was altered to include the use of a cotton liner bag where hessian handling bags are used so as to reduce the risk of transfer of ectoparasites.

5.9.3. Results

The fleas and ticks from a total of 103 animals have been examined and are detailed in Table 5.9.1. Two woylies had two species of flea; all other records were of single-species infestations of fleas and/or ticks. Most samples examined to date have come from woylies (*Bettongia penicillata ogilbyi*). A small number of samples from chuditch (*Dasyuris geoffroii*), quenda (*Isoodon obesulus*) and koomals (*Trichosurus vulpecula*) have also been examined. The flea species identified using a key from Dunnet and Mardon (1974) have been *Echidnophaga myrmecobii, Stephanocircus dasyuri and Pygiopsylla hilli.* The records of *E. myrmecobii* on one woylie and *S. dasyuri* on the koomal are new host records. Tick samples have been dominated by an *Ixodes* tick that appears not to have been described before; as well as *Ixodes tasmani, I. australiensis and I. myrmecobii.* The reference used for tick identification is Roberts (1970). Ectoparasite host records have been checked at the Australian Faunal Directory (ABRS, 2007) online.

Tables 5.9.2 and 5.9.3 list the locations of the ectoparasite samples examined to date for woylies and koomals.

Table 5.9.1. Ectoparasite list by host.

Ectoparasite species	Number of hosts with ectoparasites			
	Bettongia penicillata	*Isoodon obesulus*	*Trichosurus vulpecula*	*Dasyurus geoffroii*
Siphonaptera				
Pulicidae				
*Echidnophaga myrmecobii (*Rothschild, 1909)	1*	1	16	2
Stephanocircidae				
*Stephanocircus dasyuri (*Skuse, 1893)	21	0	1*	1
Pygiopsyllidae				
*Pygiopsylla hilli (*Rothschild, 1904)	42	0	0	0
Arachnida				
Ixodidae				
Ixodes tasmani (Neumann, 1899)	0	0	3	0
Ixodes australiensis (Neumann, 1904)	2	0	0	0
Ixodes myrmecobii (Roberts, 1962)	2	0	0	0
Ixodes spp (new)	14	0	1	0

(*represents new host record)

Table 5.9.2. Ectoparasites by location and number of animals (n) in koomals.

Site	Ectoparasite species
Balban	*Echidnophaga myrmecobii (1), Ixodes tasmani (1)*
Corbal	*Stephanocircus dasyuri (1)*
Boyicup	*Echidnophaga myrmecobii (1)*
Chariup	*Echidnophaga myrmecobii (2)*
Keninup	*Echidnophaga myrmecobii (1)*
Yendicup	*Echidnophaga myrmecobii (1)*

Table 5.9.3. Ectoparasites by location and number of animals (n) in woylies.

Site	Ectoparasite species
Balban	*Stephanocircus dasyuri (3), Pygiopsylla hilli (11), Ixodes spp (2),*
Boyicup	*Stephanocircus dasyuri (1)*
Chariup	*Stephanocircus dasyuri (1)*
Keninup	*Stephanocircus dasyuri (15), Pygiopsylla hilli (20), Ixodes spp (12))*
Warrup	*Pygiopsylla hilli (5)*
Yendicup	*Stephanocircus dasyuri (1), Echidnophaga myrmecobii (1)*
Winnejup	*Pygiopsylla hilli (1)*
Karakamia	*Ixodes spp (3)*

5.9.4. Discussion

This study has added to the host records for a number of ectoparasite species. The helmeted flea *Stephanocircus dasyuri* has been described from more than 30 mammal species in Australia; mostly small marsupials. It has been recorded on *Bettongia penicillata* in WA and *Isoodon obesulus*, *Dasyurus and Pseudocheirus* species in eastern Australia (Dunnett and Mardon, 1974) but not to our knowledge in *Trichosurus vulpecula*. The sampling of mobile ectoparasites contains potential error where handling bags are re-used between animals and traps are re-used day after day. Examination of the trapping records showed that all previous animals handled that day and trapped in that location during the trapping session were koomals therefore it is unlikely that this new host record reflects transference; rather that is indeed a new host record. Alteration to the trapping technique have included the use of a cotton liner bag where Hessian handling bags are used and a careful examination and shaking out of handling bags between animals to reduce transference of ectoparasites.

The stickfast flea *Echidnophaga myrmecobii* has a wide range of hosts in Australia including *Bettongia lesueur* from Kojonup in 1904 (Dunnett and Mardon, 1974) *Trichosurus vulpecula* (Viggers and Spratt, 1995), rodents and the European fox but not *Bettongia penicillata*. In contrast, *Pygiopsylla hilli* is confined to coastal southwestern WA and has been described from two hosts: *Bettongia penicillata* and the ngwayir, *Pseudocheirus occidentalis (*Dunnet and Mardon, 1974).

Using the tick key developed by Roberts (1970) the unidentified *Ixodes* tick keys out as *Ixodes victoriensis,* however it differs in several features from this tick in particular in the shape of the scutum, and *I victoriensis* has only been described from wombats in eastern Australia. The tick has armed (spurred) coxae and the enlarged first palp typical of female ticks of the endopalpiger subfamily. Work on identification has commenced. Of the other ticks to date, *Ixodes tasmani* is common and widespread across Australia, especially on Phalangeridae and dasyurids; *Ixodes australiensis* is described from hosts in Tasmania and southwest WA including *Bettongia* and *Ixodes myrmecobii* has been only been recorded from southwest WA, in domestic animals and *Bettongia penicillata.*

5.9.5. Future work

Several hundred specimens, including lice and mites, await identification to species level. Arrangements have been made to collect additional samples from populations in Karakamia, Dryandra, Batalling and South Australia to allow a more thorough description of ectoparasite biodiversity in the woylie and its co-habiting species. Ectoparasite burden is linked to negative effects on host fecundity, health and survival in many species of animals and birds and data from this project will be used to examine features of the host-parasite relationship in the woylie.

The pathogenicity of most haemoparasites of Australian mammals is not known (Clark, 2004), nor is the prevalence and impact of many other infectious agents. Many pathogens which have little or no effect on healthy animals in their normal host species may cause disease in individuals which are immunocompromised or stressed in some other fashion, and may have dramatic and devastating effects on naive populations. Trypanosomes and *Rickettsia* are both transmitted by ectoparasites and are proposed as the initial focus of interest as some species are pathogenic when they move beyond their normal host species or cause zoonosis (Graves *et al.*, 2006; Laakonen, 2002). Some of the authors are involved in a project examining the parasite fauna of a range of small mammals across the State. Trypanosomes have recently been detected in woylies in Karakamia as part of this sampling (unpublished data) and molecular characterisation has commenced. Further sampling will be necessary to elucidate the prevalence and nature of *Rickettsia* and trypanosomes in woylies and sympatric species before any role they may have had in the woylie decline is elucidated. DNA will be extracted from a variety of ectoparasites to examine for the presence of trypanosomes and *Rickettsia* to explore their modes of transmission.

5.9.6. Conclusion

Relatively little is known about the ectoparasite fauna of mammals in Western Australia and their potential to act as vectors of disease. The ectoparasite identification keys used were published more than 30 years ago and tend to reflect a sampling bias towards eastern Australia: (for example *Pygiopsylla hilli* was previously described from only 5 specimens collected from woylies and ngwayir in southwest WA). Host records are reflective of effort by researchers and the large numbers of ectoparasite samples being collected make it likely that further new species of ectoparasites will be found and host records expanded. Information on ectoparasite biodiversity can help predict spread of novel vector-borne diseases including zoonosis, and can aid in translocation and reintroduction programs in animals such as woylies.

An examination of any role that disease may have had in the woylie decline is likely to unearth new infectious agents as relatively little is known about the pathogens of Australian mammals. An important first step is to survey for potentially pathogenic organisms in woylies and other mammals that share their environment to which end work on trypanosomes and *Rickettsia* has commenced.

5.9.7. Acknowledgments

Russ Hobbs[1], Aileen Elliot[1] and Yazid Abdad[1] for assistance with ectoparasite identification.

5.9.8. References

Australian Faunal Directory. Australian Biological Resources Survey, Canberra. Accessed 23 September 2007. http://www.environment.gov.au/biodiversity/abrs/online-resources/fauna/afd/index.html

Dunnet, G.M. and Mardon, D.K. (1974). A monograph of Australian Fleas (Siphonaptera). Australian Journal of Zoology Supplementary Series. 30 1-273

Clark, P. (2004) Haematology of Australian Mammals. CSIRO Publishing

Graves, S., Unsworth, N and John Stenos. (2006) Rickettsioses in Australia Annals of the New York Academy of Science 1078: 74–79

Hamilton, PB, Stevens, JR, Holz, P, Boag, B, Cooke, B, Gibson, W. (2005) The inadvertent introduction into Australia of Trypanosoma nabiasi, the trypanosome of the European rabbit (Oryctolagus cuniculus), and its potential for biocontrol. Molecular Ecology 14:3167-3175.

Hoodless, A. N., K. Kurtenbach, P. A. Nuttall and S. E. Randolph (2002) The impact of ticks on pheasant territoriality Oikos, 96, 245.

Khokhlova, I. S., B. R. Krasnov, M. Kam, N. I. Burdelova and A. A. Degen (2002) Energy cost of ectoparasitism: the flea Xenopsylla ramesis on the desert gerbil Gerbillus dasyurus Journal of Zoology, 258, 349.

Laakkonen (2002) Trypanosome parasites in the invading Rattus rattus and endemic rodents in Madagascar. In Rats, Mice and People: Rodent Biology and Management (eds Singleton, G., Hinds, L., Krebs, C. and Spratt, M.) ACARI Canberra.

Mathews, F., Moro, D., Strachan, R., Gelling, M. and Buller, N. (2006) Health surveillance in wildlife reintroductions. Biological Conservation 131, 338-347.

Roberts, F.H.S. (1970) Australian ticks (CSIRO: Australia).

Viggers, K. L., D. B. Lindenmayer and D. M. Spratt (1993) The Importance of Disease in Reintroduction Programmes. Wildlife Research, 20, 687.

Viggers, K. L and D. M. Spratt (1995) The parasites recorded from Trichosurus species (Marsupialia: Phalangeridae) Wildlife Research, 22, 311-32

Whiteman, NK, and Parker, P.G. (2004) Body condition and parasite load predict territory ownership in the Galapagos Hawk Condor 106 (4): 915-921

5.10. Bacteriology

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Abstract

Faecal and ectoparasite samples from woylies and other co-habiting native species were analysed for the presence of the potential bacterial pathogens *Salmonella* and *Rickettsia.* This work constitutes a component of the disease investigations within the Woylie Conservation Research Project (WCRP) - diagnosing recent declines in woylie populations in southwestern Australia. The significance of the bacterial isolates will be discussed in relation to declining woylie populations and zoonotic infections.

5.10.1. Introduction

After consideration by the Woylie Disease Reference Council (WDRC), and without any evidence to support the possibility of bacterial infections as the cause of the woylie decline, a decision was made to concentrate on two relatively non-invasive sample types to investigate the carriage of potential bacterial pathogens by woylies. The decision was influenced by samples already being collected as part of the current research, which included (i) faecal samples and (ii) ectoparasite samples. In particular, the enteric samples were to be analysed for the presence of *Salmonella*, which are potentially pathogenic bacteria responsible for disease in a wide range of domestic and wild animals. The ectoparasite samples (principally ticks) were to be analysed for the presence of members of the genus *Rickettsia*, which although not generally believed to be pathogenic for wildlife, are nonetheless zoonotic, i.e. they can potentially infect humans and which might under certain circumstances cause disease in other species.

5.10.2. Results

5.10.2.1. *Rickettsia*

Trapped woylies and other co-habiting species were examined carefully for ectoparasites, which were removed using forceps and/or flea combs and placed alive in sealed, plain vials. These were subsequently identified using taxonomic keys.

A total of 23 tick samples were screened for *Rickettsia* DNA via PCR, resulting in four positives in one tick species, and two other tick species coming up negative. 4/16 *Amblyomma triguttatum* were positive for a spotted fever *Rickettsia*, most likely candidatus "*Rickettsia gravesii*" which has been found in a number of animal species in the region. Six *Ixodes australiensis* and one *Ixodes fecialis* were also screened but were negative (Table 5.10.1).

Rickettsia screening summary

Prevalence in *A. triguttatum*

Total number screened = 16

Total positives = 4

Prevalence = 25%

Rickettsia species = Candidatus "*Rickettsia gravesii*" – to be confirmed

Samples tested were from woylie, koomal (common brushtail possum, *Trichosurus vulpecula*) and human

Woylie Samples = 16

2/11 *A. triguttatum* (~20%)

0/5 *I. australiensis*

Koomal Samples = 6

1/4 *A. triguttatum* (25%)

0/1 *I. australiensis*

0/1 *I. fecialis*

Human Samples = 1

- 1/1 *A. triguttatum* (100%)

Table 5.10.1. Distribution of *Rickettsia gravesii* in samples screened for the Woylie Conservation Research Project.

Location	Host Species	Number (+ve / # screened)	Tick Species
Warrup	Woylie	0/5	*I. australiensis*
	Koomal	0/1	
Winnejup	Koomal	0/1	*I. fecialis*
Corbal	Human	1/1	*A. triguttatum*
	Koomal	1/4	
	Woylie	1/8	
Dryandra	Woylie	1/3	*A. triguttatum*

Of interest is that a novel species of tick, belonging to the genus *Ixodes*, has been observed from the woylies, further work is being done to characterise it.

5.10.2.2. Salmonella

Fresh faeces or faecal swabs were collected from woylies and any other species trapped within the Upper Warren in March-April 2006. *Salmonella* tests were conducted under contract by Pathwest. A total of 233 faecal samples were screened for *Salmonella*, resulting in 19 positives from four animal species, namely woylies (5/137; Table 5.10.2), koomal (2/74; Table 5.10.3), chuditch (10/15; Table 5.10.4) and quenda (2/7; Table 5.10.5). The 19 positive samples consisted of 15 different serovars, the most numerous of which were *Salmonella* Bootle and *S*. O group 1 (Table 5.10.6).

Salmonella screening summary:

Total samples tested = 233

Total samples positive = 19

Total prevalence in medium-sized mammals = 8.15%

Woylie (positive/total sampled) = 5/137 (3.65%) 1 woylie had dual infection with 2 serovars

Koomal (positive/total sampled) = 2/74 (2.7%)

Chuditch (positive/total sampled) = 10/15 (66.7%) 1 chuditch had dual infection

Quenda (positive/total sampled) = 2/7 (28.6%) 1 quenda had dual infection

Table 5.10.2. Woylie faecal samples tested for *Salmonella* at Upper Warren forest blocks.

Location	# tested	# positives	Salmonella ID
Winnejup	10	0	
Warrup	27	1	*S*. Choleraesuis var Australia
Boyicup	4	0	
Chariup	4	0	
Balban	45	2	*S*. Bootle (47:k:1,5) / *S*. Eastbourne (9,12:eh:1,5) / *S*. Charity
Corbal	26	1	*S*. Lindern
Keninup	16	1	*S*. Infantis
Yendicup	5	0	

Table 5.10.3. Koomal faecal samples tested for *Salmonella* at Upper Warren forest blocks.

Location	# tested	# positives	Salmonella ID
Winnejup	3	0	
Warrup	6	0	
Boyicup	5	0	
Camelar	5	0	
Chariup	11	1	*S*. Sachsenwald
Balban	9	0	
Corbal	10	0	
Keninup	6	0	
Moopinup	9	1	*S*. Chester
Yackelup	6	0	
Yendicup	4	0	

Table 5.10.4. Chuditch faecal samples tested for *Salmonella* at Upper Warren forest blocks.

Location	# tested	# positives	Salmonella ID
Winnejup	1	1	*S*. Wandsbek
Warrup	2	0	
Balban	2	1	*S*. Bootle (47:k:1,5), *S*. Orion (3,10:y:1,5)
Corbal	2	0	
Keninup	7	7	*S*. Muenchen (2), *S*. Bootle, *S*. Arizona ss III, *S*. Houten. *Salmonella* O group C (6,7:c:-), *Salmonella* Orion
Moopinup	1	1	*S*. Houten (43:z4,z23:-)

Table 5.10.5. Quenda faecal samples tested for *Salmonella* at Upper Warren forest blocks.

Location	# tested	# positives	Salmonella ID
Boyicup	3		0
Camelar	3	2	*S.* Birkenhead (6,7:c:1,6), *S.* O group I (16:-:-) (both)
Moopinup	1	0	

Table 5.10.6. Distribution of *Salmonella* serovars in the Upper Warren.

Location	Number	Serovar	Host Species
Keninup	1	*S.* Arizona sub species III	Chuditch
Camelar	1	*S.* Birkenhead	Quenda*
Balban, Keninup	3	*S.* Bootle	Chuditch (2)*, Woylie*
Balban	1	*S.* Charity	Woylie
Moopinup	1	*S.* Chester	Koomal
Warrup	1	*S.* Choleraesuis	Woylie
Balban	1	*S.* Eastbourne	Woylie*
Keninup, Moopinup	2	*S.* Houten	Chuditch
Keninup	1	*S.* Infantis	Woylie
Corbal	1	*S.* Lindern	Woylie
Keninup	2	*S.* Muenchen	Chuditch
Balban, Keninup	2	*S.* Orion	Chuditch*
Chariup	1	*S.* Sachsenwald	Koomal
Winnejup	1	*S.* Wandsbek	Chuditch
Keninup, Camelar	3	*Salmonella* O group (1 and C)	Chuditch, Quenda (2)*

*co-infection with other *Salmonella* serovar

5.10.2.3. Interpretation of results

Although only four isolates of *Rickettsia* were identified from 23 ticks analysed, it is highly likely that many of the other ticks were infected as high prevalences have been found in ticks from other animal species, e.g. *Antechinus* and feral pigs, in similar habitats (Owen *et al.*, 2006). The *Rickettsia* belonged to the Spotted Fever Group of the genus, which contains many human pathogens, including at least two in Australia that cause 'Australian spotted fever' and 'Flinders Island spotted fever'. While this *Rickettsia* was not identified to the species level, recent work by the Veterinary Public Health group at Murdoch University has identified a hitherto unknown species of SFG *Rickettsia* in a wide range of native animals, tentatively named *R. gravesii*. This may be the species found in the tick from the Upper Warren although this is yet to be confirmed by DNA sequencing. Of interest is that another novel SFG species, tentatively named *'R. antechini'* was identified from ticks off *Antechinus* in Dwellingup (Owen, PhD thesis, 2007), thus a possibility exists that the SFG *Rickettsia* from the woylies is also a novel species.

With respect to *Salmonella*, the most interesting fact was the wide variety of serovars recovered, possibly reflecting the different parts of the ecosystem that the four species of animals inhabit and their dietary habits. For example, koomal, which are largely arboreal, had the lowest number of *Salmonella* strains (and prevalence) recovered, followed by woylies (which have a narrow dietary range), then quenda which are more omnivorous and then the carnivorous chuditch. The high prevalence of *Salmonella* in chuditch is speculated to reflect their dietary habits in which a range of birds, small mammals, reptiles and carrion might play a part. The large variety of serovars also reflects the widespread nature of this organism in a forest ecosystem, with possible sources of infection being faecal-contaminated food and water supplies. Although kangaroos were not sampled in these areas, a similar wide range of serovars has been found in these animals in studies carried out in other ecosystems, including southwestern Australia (unpublished data). A number of the serovars are found in human infections, with humans possibly contributing to the environmental contamination either directly or indirectly via water.

5.10.2.4. Involvement of *Rickettsia* and Salmonella in woylie declines

The possible involvement of *Rickettsia* in the woylie decline is remote, as despite being commonly associated with ticks from wildlife, there has been no evidence of these organisms affecting native animals in other habitats or ecosystems worldwide. Nevertheless, occasional infections have been documented in domestic dogs (Solano-Gallego *et al.*, 2006) and information on their possible effect on animals under severe stress is unknown.

Similarly, although *Salmonella* have also been shown to be commensals in the intestines of a wide range of native animals in Australia (Bensink *et al.*, 1991; Hart *et al.*, 1985; Thomas *et al.*, 2001), little evidence is available to support their role as pathogens and their involvement with the woylie decline is also believed to be remote, particularly as the *Salmonella* obtained were from samples collected from trapped healthy animals. This data shows a prevalence of *Salmonella* in the marsupials, however gives us no evidence of any effects they may have on their hosts' well-being.

Salmonellosis has previously been documented as both the primary cause of death (Atkinson, 1964; Mushin and Ashburner, 1964), and as a secondary invader (Winter and O'Connor, 1957) in macropods that died with enteritis from zoos or private collections. However, whilst all *Salmonella* species are capable of causing disease, they are more commonly found in the intestinal tracts of healthy, rather than sick or diseased animals (Samuel, 1983). Additionally, once introduced to a group of macropods, *Salmonella* is capable of persisting for long periods of time, but whether they are continually cycled through the population, or if particular individuals remain carriers for a long time is not known (Samuel, 1983).

Nevertheless, of the two organisms, *Salmonella* is well recognised as an important pathogen of many domestic animal species and people, particularly in animal populations under stress (dietary, climatic, etc.) and the role of the organism as a woylie pathogen should not be discounted entirely. However, a relatively high prevalence of *Salmonella* within a population makes it difficult to judge the significance of their isolation from sick or dead animals. Evidence to support the role of *Salmonella* as a pathogen however would most likely come from its isolation from the internal organs of sick or recently dead animals and the absence of other likely causes of disease.

5.10.3. Recommendations and future research

While there is probably no reason to further research these organisms for their involvement in the woylie decline, their role as pathogens for occupational and recreational groups active in native forests would support further work into their ecology and epidemiology. As the Murdoch *Rickettsia* research group is actively investigating the diversity and importance of *Rickettsia* in WA it would be appreciated if collection of ectoparasites from trapped native animals was continued, as this is a simple, non-invasive activity, which will add considerably to our understanding of this fascinating group of organisms.

While only members of the genus *Rickettsia* were targeted in the ticks, it would be of interest to investigate the presence of other potential pathogens in ectoparasites, for example *Bartonella* spp in fleas, and this may be incorporated into a PhD project started in 2007. In addition to the novel *Rickettsia* identified from Antechinus ticks, Owen (PhD thesis, 2007) also identified a novel *Bartonella* species from fleas off the same animals. Their involvement in any disease process however was not investigated. *Bartonella* species are known to cause disease occasionally in dogs and people so further investigation of these organisms in fleas from woylies may be justified.

Although it would also be of immense value to further investigate *Salmonella* in native animals from a scientific and ecological perspective, at this stage the costs involved in collection and analysis of samples prohibits this activity. However, any faecal samples sent for analysis for bacterial pathogens in the future should include screening for *Salmonella*, and all isolates recovered should be stored for further characterisation.

There are a whole host of bacterial pathogens that exist in the native Australian fauna some of which have no doubt been around since before the arrival of settlers and others which were probably introduced as a result of the introduction of non-native animals into the wild. Most of the bacterial flora, however, do not pose a significant threat other than to the individual animal as a secondary infection. However, there are a few that would be worth considering in future investigations, one is “lumpy jaw” or necrobacillosis, caused by the bacteria *Fusobacterium necrophorum* and possibly *Actinomyces* spp. and *Bacteroides* spp. in the absence of *F. necrophorum*. Lumpy jaw is usually regarded as a wound infection and is associated with the presence of a specific bacteria in large numbers on the ground or pasture. Other associated bacterial infections include *Mycobacterium* and *Clostridium tetani* (cause of tetanus), the former only recorded in captive macropidae and the latter reported sporadically in northern Australia (Speare *et al.,* 1989).

5.10.4. References

Atkinson, N (1964) “Salmonellosis in Australia” in the book “The World Problem of Salmonellosis”: 539-589.

Bensink, JC, Ekaputra, IGMA and Taliotis, C (1991) The isolation of salmonella from kangaroos and feral pigs processed fro human consumption. Australian Veterinary Journal, **68**: 106-107.

Hart, RP, Bardshaw, D and Iveson, JB (1985) *Salmonella* infections in a marsupial, the quokka (*Setonix brachyurus*), in relation to seasonal changes in condition and environmental stress. Applied and Environmental Microbiology, **49**: 1276-1281.

Mushin, R and Ashburner, F (1964) Ecology and epidemiology of Coliform infections I. The incidence of enteropathogenic and other specific serotypes of Escherichia coli. Medical Journal of Australia, **1**: 257-262.

Owen, H. (2007). Murdoch University, PhD thesis ‘Detection and characterization of rickettsiae in Western Australia’

Owen, H, Clark, P, Stenos, J, Robertson, I and Fenwick, S (2006) Potentially pathogenic spotted fever group rickettsiae present in Western Australia. Australian Journal of Rural Health, **14**: 284-285.

Samuel, JL (1983) “Salmonella in macropods” in the book “Wildlife Diseases of the Pacific Basin and Other Countries”: 61-63.

Solano-Gallego, L, Kidd, L, Trotta, M, Di Marco, M, Caldin, M, Furlanello, T and Breitschwerdt E (2006) Febrile illness associated with *Rickettsia conorii* infection in dogs in Sicily. Emerging Infectious Disease, **12**: 1985-1988.

Speare, R, Donovan, JA, Thomas, AD and Speare, PJ (1989) “Diseases of free-ranging macropodoidea” in the book “Kangaroos, Wallabies and Rat-Kangaroos”: 705-734.

Thomas, AD, Forbes-Faulkner, JC, Speare, R and Murray, C (2001) Salmonelliasis in wildlife from Queensland. Journal of Wildlife Disease, **37**: 229-238.

Winter, H and O’Connor, RF (1957) *Salmonella birkenhead* from a kangaroo. Australian Veterinary Journal, **33**: 67.

See Note (at end of this document) “General subjects and structure for subsections reporting on results”

5.11. Identification of potential diseases and risk assessment in relation to recent woylie declines

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Abstract

A qualitative approach has been used in order to assess disease risks related to declines in woylie populations in southwestern Australia. Certain disease agents have been recognised as critical to this risk assessment including: Chlamydiales, Macropod Herpesvirus, Orbivirus, Encephalomyocarditis virus and *Neospora caninum*.

Arrangements for the analysis of these diseases are in progress. The outcomes of this investigation are expected to assist the understanding of the woylie decline dynamics.

5.11.1. Introduction

The role of disease as a contributing or determining factor in wildlife decline or extinction is being increasingly recognized (Spalding and Forrester, 1993; Daszak and Cunningham, 1999; Daszak *et al.,* 2000; Spielman, 2001). In fact, it has been suggested that disease screening should be included in wildlife monitoring program and conservation projects (Spalding and Forrester, 1993; Aguirre *et al.,* 2002).

A significant challenge for this process is the paucity of known information on diseases in wildlife and the almost complete absence of baseline data to determine what is considered as 'normal' (Spalding and Forrester, 1993). Additional complications are generated by financial constraints and practical limitations. Moreover, diagnostic techniques are not always available for the species being investigated and use of tests developed for other species is not always reliable.

Therefore, it is necessary to use a logical and scientifically based process to address these limitations and develop a realistic solution that provides a comprehensive view of this important issue.

Using models proposed in the literature, especially for wildlife translocations, we have followed a systematic approach in order to be able to establish which diseases have to be prioritized in further investigations (Spalding and Forrester, 1993; Jacob-Hoff *et al.,* 2001; Munson and Karesh, 2002; Armstrong *et al.,* 2003).

5.11.2. Methods

As a quantitative evaluation of the disease risk is not achievable due to lack of knowledge of disease prevalence and species susceptibility, a qualitative approach has been used. Jacob-Hoff *et al.* (2001) showed that the qualitative disease risk analysis is a practical and useful tool to achieve our aims. As suggested by Munson and Karen (2002), we took into consideration important diseases reported to have occurred in bettongs and other macropods known to co-habit in the same geographic area. This has been accomplished through a broad literature review and the acquisition of unpublished critical data from the Australian Wildlife Health Network (AWHN).

For the sake of brevity we omitted in this section the pathogens that are currently being investigated within the Woylie Conservation and Research Project (see relevant sections in this document). Once we created a list of diseases that we believed were important and merited special consideration, we established specific criteria to rank them. Factors that were considered in assessing the importance of a given disease included:

- Species reported to be affected by the disease
- Known presence of the disease (in captive populations and wild populations)
- Mode of transmission
- Outcome of infection (e.g. clinical signs, pathologic lesions)
- Pathogenicity of the disease (on its own accord, in synergy with other pathogens)

A qualitative grading system was applied to these factors to determine the significance of the disease in relation to population declines, and therefore assist in guiding future directions for disease investigations for this program.

5.11.3. Results

The list of selected disease agents considered to affect woylies is summarized in Table 5.11.1-3.

Investigations into haemoparasites, endoparasites, ectoparasites, and *Toxoplasma gondii* are already in place (see relevant sections in this document) and will not be discussed further in this section. Besides, a preliminary screening for Salmonellosis has been carried out (see relevant section in this document). Thus, this agent is no longer mentioned in this section.

Diseases considered to be significant for future investigations in this program include:

- Chlamydiales
- Macropod Herpesvirus
- Macropod Orbivirus
- Encephalomyocarditis virus
- *Neospora caninum*

These pathogens are considered to have potential for rapid and widespread population declines based on the outcomes of this risk assessment process.

Arboviruses have been reported to occur in macropods, although have not been reported to have shown clinical effects of these infections. These viruses are an important potential zoonosis and therefore may also warrant further investigation.

Certain disease agents have been ranked as a low or very low priority because they have not been identified at all or rarely reported to occur in Western Australia. These include: *Clostridium tetani*, *Leptospira interrogans*, *Pseudomonas pseudomallei*, *Coxiella burnetti*, *Rickettsia rickettsii*, *Leishmania* spp, Adenoviruses and 'wobbly possum syndrome'. A number of other pathogens are considered to be opportunistic or secondary infections and unlikely to be responsible for population declines in their own right. These include: *Pseudomonas* spp, *Escherichia coli*, *Klebsiella* spp and *Clostridium piliforme* (Tyzze's disease). Other diseases, such as Lumpy jaw, Mycobacteriosis, and a number of fungal diseases, are considered chronic diseases and would result in different decline patterns and observations than those seen with the current situation.

5.11.4. Discussion

Of the diseases considered important for further investigations through this risk analysis process, Macropod Orbivirus infection would be considered a priority. Infection by Orbiviruses, especially of the Wallal and Warrego serogroups, has been reported in several species of macropods that are closely related to woylies, with serious clinical consequences and an Orbivirus, probably from the Eubanangee serogroup has been associated with a sudden death syndrome in captive tammar wallabies from several research facilities and zoological gardens in NSW and Queensland. (Table 5.11.2.) (Rose *et al.*, 1999, Daszak *et al*., 2000). With the wild populations disease prevalence rates were not able to be determined but the Kangaroo blindness syndrome was noted to be widespread over a large geographical area and with the sudden death syndrome in captive tammar wallabies there was a high disease incidence rate in some collections. Thus, woylies may be considered to be potentially susceptible to Orbivirus pathogens. In its extreme, a severe clinical form, similar to that described for other macropods, could have critical consequences in woylie populations. A number of epidemics of this disease have been reported in wild populations of macropods in Australia between 1969 and 1996, including outbreaks from the South West region of Western Australia (Hooper 1999; Hooper *et al.,* 1999). Hooper *et al.* (1999) also reported a high seroprevalence for this virus in wild kangaroos and wallabies throughout the region. The virus has been isolated from eye and brain tissues collected from kangaroos near Albany, Esperance and Perth (Hooper *et al.,* 1999). Consequently, it can be concluded that the virus is well established in the region.

Macropod Herpesvirus (MHV) is another viral infection which is known to be present in the region. MHV has resulted in sudden death in captive populations of dorcopsis wallabies, quokkas, western grey kangaroo and woylie (Dickson *et al.,* 1980; Callinan and Kefford, 1981; Wilks *et al.,* 1981). Antibodies have been found in both wild and captive populations of macropods - a study of antibody levels to MHV by Webber and Whalley (1978) indicated a higher prevalence of antibody in captive populations, whilst the highest levels were found in a population of tammar wallabies experiencing an outbreak of MHV. High antibody levels in captive animals may reflect a higher level of virus transmission due to crowding, increased contact rates with infected animals, or to conditions of stress leading to expression of latent virus (Webber and Whalley, 1978). In this regard, the fact that woylie populations started declining when they reached the highest density would suit the ecology of this infection. However, the widespread distribution of antibody levels, as discussed by Webber and Whalley (1978), suggests that this virus has evolved with marsupial species and may be endemic in wild populations. Herpesvirus can, in some instance produce severe acute disease with mortality, especially in young animals and with some herpesviruses (e.g. Aujeszky's disease virus, herpes B virus of macaques, alcephaline herpesvirus 1) after introduction into a new species (Murphy *et al.,* 1999). Dickson *et al.* (1980) reported on an outbreak of MHV infection that resulted in the acute deaths of eight woylies, among other species, over a period of a week. This report indicates that woylies may be highly susceptible to MHV infection but with opportunistic surveillance as conducted with woylie populations, it may be difficult to detect presence of Herpesvirus disease in a population. Clinical symptoms, such as ulcerations or vesicles, may be rare and difficult to detect in field investigations. In this scenario, affected population could undergo a steep decline, with increased mortality after introduction of the virus, similar to what the available monitoring data suggests. Further investigation would initially require examination for serological evidence of MHV infection in woylie serum collection.

Chlamydiales are bacteria known to cause significant disease in other marsupials. In fact, it has been proven that they cause conjunctivitis and proliferation of the eyelid in western barred bandicoot and greater glider (Bodetti *et al.,* 2003), and conjunctivitis, keratitis, cystitis as well as infertility in koalas (Fowler *et al.,* 2003). In addition, Chlamydiales types are known to be present in the region because they have been isolated in a number of species. For example, the bacteria has been isolated from western barred bandicoot and greater bilby from Dryandra, and gilberts' potoroo from Albany (Bodetti *et al.,* 2003). However, the clinical significance in macropods and especially the possible role of this pathogen in the woylie decline is still unclear. The lack of information about this pathogen in the affected woylie populations is a significant gap in determining the importance of these agents.

Encephalomyocarditis virus is a known pathogen in rural areas in Australia and it can occur when rodents build up to plague proportions around piggeries. The disease in pigs is acute with sudden death or acute neurological signs. If woylies are susceptible to this virus, it could have serious consequences for the species. Consequently, we considered that it would be worth testing the woylie groups for exposure to this virus.

Neospora caninum is a protozoa of recent discovery (first identified in 1984) (Bjerkås *et al.,* in Reichel, 2000). It is now described world-wide and isolated from a variety of species including dogs, cattle, sheep, goats, deer and horses (Reichel, 2000). We are not aware of its clinical significance in wildlife but considering the seriousness of the clinical signs (paresis or paralysis in dogs and abortion in cattle), we believe that it would be worthwhile to further investigate the possible prevalence of this infection in woylie populations. The possibility to adapt existing serological tests to woylies is being explored.

Arboviruses comprise mosquito-born viruses including Flaviviruses (Murray Valley Encaphalitis and Kunjin are present in northern WA) and Alphaviruses (Ross River Virus and Barmah Forrest Virus are present in WA). Some of the infections caused by those viruses are important zoonoses and they are worth some special considerations. Macropods have been infected but not shown any evidence of disease with the Flaviviruses or Alphaviruses and from sero-epidemiological studies appear to be possible virus reservoirs (Russell, 2002; Old and Deane, 2005). Although it is not expected that either of these viral genuses would be causing disease problems in woylie, knowing whether this species of macropods is contributing to maintain the virus in the habitat would have a great interest in terms of human health. Moreover, a positive serologic result could give an indication on the exposure of this species to biological vectors such as mosquitoes.

Two other viruses affecting European rabbits that are present in Western Australia, Rabbit Calicivirus and Myxomatosis virus, were also considered in relation to possible effects on woylies and were discounted. Both Rabbit Calicivirus and Myxomatosis viruses are very host specific; had been evaluated in a wide range of Australian wildlife before use for biological control of European rabbits and since release have not been found to infect animals other than rabbits.

5.11.5. Future work

Contact with Dr Cheryl Johansen, who leads the Arbovirus surveillance project at UWA, Department of Microbiology, has been established and arrangements made for an initial screening of Alphaviruses and Flaviviruses in around 200 woylie serum samples. Should the Woylie Disease Reference Council (WDRC) decide to progress with the investigation of these viruses, the testing should most likely start by the end of 2007.

Currently, there are no virus laboratory facilities available at Murdoch University for Orbivirus and possibly Herpesvirus testing. The opportunity of temporarily using existing facilities for virus neutralisation tests (VNT) for these viruses and/or collaboration with external facilities are being explored.

We are currently seeking more information regarding Chlamydiales infection in macropods and a request has been forwarded to the AWHN. This could give access to unpublished data which may provide critical information and help in deciding whether and which test should be run for this group of pathogens.

We are searching for updated epidemiological data on *Neospora caninum* in livestock and availability of serological tests, in order to discuss this issue in the next WDRC meeting.

The use of population viability analysis (PVA) integrating the results of the ongoing disease investigations has been suggested (Haydon *et al.,* 2002; Miller, 2003) and we believe that it could provide useful information to assist in the identification of the cause(s) of woylie decline as well as support management decision.

5.11.6. Conclusion

A qualitative approach, as suggested by the literature, has been used in order to assess the disease risk for a specific geographic area and for a specific species. We have been able to create a list of known diseases and, according to the available information, prioritise those considered most likely to contribute to patterns of population decline observed in woylies in southwestern Australia. One of the main strengths of this process has been the multidisciplinary collaboration. In fact, the involvement of scientists from different areas of expertise overcame the risk that a personal point of view and/or experience would become the main reasoning of the assessment.

The outcome of this collegial work is that “new” diseases in addition to those that have been already tested during the first phase of the Woylie Conservation Research Project have been highlighted on the basis of the information reported in the literature and recent outcomes of the fieldwork.

These tests may reveal if there are some association between the seroprevalence of the selected viruses in the declining population compared to the healthy population, however, it will still be necessary to detect the viruses in affected animals before a role for the virus can be clearly established. Nevertheless, we believe that this investigation will obtain valuable information in regards of the woylie decline, and also baseline data for future monitoring and management activities for woylies and other sympatric species.

The results of this assessment will be discussed during the next WDRC meetings, along with the information regarding available tests and potential collaborations. The council will come to a common agreement on how to progress in this important area of investigation.

Table 5.11.1. List of selected diseases: bacteria. *Not clearly reported in the literature. ^Not sufficient data available.

	Disease	Lumpy jaw	Mycobacterium spp	Tetanus	Salmonellae	Leptospirosis	Listeria	Clostridium piliforme (Tyzzer's disease)	Pseudomonas pseudomallei (Burkholderia pseudomallei)	Pseudomonas pyocyanea	Pseudomonas spp	Escherichia coli
	Species reported	Macropodoidea	Macropodoidea	Macropodoidea	Macropodoidea	Macropodoidea	Macropodoidea	Possums,dasyurids, Wombat, koala	Tree-kangaroos	Possums, macropods, koala	Macropodoidea	Macropodoidea
Known Presence	**Captive Pop's**	Yes	Yes, including Woylie	*	Yes	Yes	Yes	Yes	*	*	Joeys	Joeys
	Wild Pop's	*	Unknown	*	Yes, but no clinical cases	Yes	Unknown	Brushtail and ringtail possum	Yes	*	*	Unknown
	Transmission			Wounds		Rodents						
	Clinical signs		Abscesses, skin ulcers, dyspnea, neurol.	Convulsions, muscle stiffness death		Uncommon		Sudden death or diarrhoea/anorexia	Melioidosis, nasal discharge		Pneumonias	Pneumonias
	Lesions					Focal interstitial nephritis	Hepatic focal Abscesses	Necrotizing hepatitis and myocarditis	Multifocal abscesses	Smelly liquid in the pouch		
	Comments	Clinical signs would be evident. Unlikely to be responsible of a so rapid decline. Body condition should be poor.	Never noted in free-ranging. Chronic disease	Uncommon in wildlife. More common in northern Australia.	Commonly isolated from macropods.	Uncommon, no clinical disease in wildlife. No identified in WA.		Wild animals from Sydney. Potentially persistent in the soil	More common in northern Australia.	Pouch are regularly checked.	Identified only in cap. animals.	Identified only in cap. animals.
	Reference	Speare *et al.* 1989	Speare *et al.* 1989	Speare *et al.* 1989, Fowler *et al.* 2003	Speare *et al.* 1989	Speare *et al.* 1989	Canfield and Hartley 1992	Fowler *et al.* 2003	Jacob-Hoff 1993, Fowler *et al.* 2003	Fowler *et al.* 2003	Jackson 2003	Jackson 2003
Pathogenic Potential	**Likely Presence**	moderate	moderate	low	moderate/high	very low	low	low	low	low	low	low
	Independent	low	moderate	high	moderate	low	moderate	moderate	low	low	moderate	moderate
	In concert	low	moderate	nil	moderate/high	low	moderate/high	moderate/high	low	low	moderate/high	moderate/high
	Risk / Priority	low	low	low	moderate/high	low	low	low	low	low	low	low

Table 5.11.1. List of selected diseases: bacteria. Cont'd *Not clearly reported in the literature. ^Not sufficient data available.

	Disease	Klebsiella spp	Yersinia pseudotuberculosis	Coxiella burneti	Rickettsia rickettsii	Chlamydiales
	Species reported	Macropodoidea	Possums	Macropodoidea	Macropodoidea	A variety of marsupials
Known Presence	**Captive Pop's**	Joeys	*	*	*	*
	Wild Pop's	*	*	Yes	Yes	Yes
	Transmission		Rodents and birds	Ticks	Ticks	
	Clinical signs	Pneumonias	Diarrhoea			Conjunctivitis, rhinitis, pneumonia, pelvic inflammatory disease, infertility.
	Lesions		Enteritis, septicaemia, hepatic , splenic and renal abscesses			
	Comments	Identified only in cap animals.		No significance in free-ranging, no ill. Queensland and Tasmania	Queensland	Isolated from wild animals from Dryandra (WB Bandicoot) and Albany (Gil.'s Potoroo). Many carried without clinical symptoms.
	Reference	Jackson 2003	Fowler *et al.* 2003	Speare *et al.* 1989	Speare *et al.* 1989	Bodetti *et al.* 2003
Pathogenic Potential	**Likely Presence**	low	low	very low	low	moderate
	Independent	moderate	moderate/high	low	moderate/high	moderate
	In concert	moderate/high	moderate/high	low	high	low-moderate
	Risk / Priority	low	low	very low	low	moderate

Table 5.11.2. List of selected diseases: virus. *Not clearly reported in the literature. ^Not sufficient data available.

	Disease Virus	Macropod Herpesvirus	Macropod Pox Virus	Arbovirus	Ross River Virus	Encephalomyocarditis Virus EMCV	Orbivirus (Wallal and Warrego serogroup)	Orbivirus (Eubenangee serogroup)	Adenovirus	Wobbly possum syndrome, envelope RNA Virus
	Species reported	Macropodoidea; wombats	Macropodoidea	Macropodoidea	Macropodoidea	Tree kangaroo; quokka	Macropodoidea	Tammar wallaby	Macropodoidea	Brush-tail possum in NZ
Known Presence	**Captive Pop's**	Yes	*	*	Yes	Yes, Tree kangaroo	*	*	*	*
	Wild Pop's	Yes. Serologically positive	Yes	Yes. Serologically positive	Yes. Serologically positive	Unknown	Yes	*	Yes, in B. gaimardi	*
	Transmission		Skin, mosquitoes		Mosquitoes	Rodents	Culicoides spp	Culicoides spp		
	Clinical signs	Conjunct., Dyspnea, vesicles and ulceration oral cav., cloacae and genital tract, death. In wallabies cases of reduce reproduction succ. (Finnie 1980)	Resemble papillomas on tail, dorsum, lip and legs		Fever, rash, polyarthritis	Sudden death, dyspnoea	Chorioretinitis, blindness, circling	Death, muscles fasciculations	None	
	Lesions	Multifocal necrosis, intranuclear inclusion bodies in various organs. Liver lesions in Woylie (Canfield and Hartley 1992).	Eosinophilic cytoplasmic inclusion bodies in epithelial cells			Pulmonary congestion and oedema, myocarditis	Nonsupperative panuveitis, retinitis	Oedema hind limbs; Haemorrhage adductors, thorax, dors cervical area and retroperitoneally; Necrosis of lymphoid germinal centres, gastric ulceration and periacinar hepatic necrosis	Enlarge epithelial cells with intranuclear inclusion bodies	
	Comments	No clinical disease reported in wild animals, but presence of sero-positive wild animals. Clinical sings should be evident.		Viraemia but not ill	Human health risk. Macropods seem to be reservoirs		Epidemics in WA (Esperance, Albany, Perth) in 94-96 (Hooper 1999)			
	Reference	Speare *et al.* 1989; Dickson *et al.* 1980; Fowler *et al.* 2003	Speare *et al.* 1989; Fowler *et al.* 2003	Speare *et al.* 1989	Old and Deane 2005	Jackson 2003; Fowler *et al.* 2003	Jackson 2003, Fowler *et al.* 2003	Fowler *et al.* 2003	Speare *et al.* 1989; Fowler *et al.* 2003	Fowler *et al.* 2003
Pathogenic Potential	**Likely Presence**	high	low	moderate/high	moderate/high	moderate/high	moderate/high	moderate/high	low	very low
	Independent	moderate	low	low	low	moderate	moderate	moderate	moderate	moderate
	In concert	moderate/high	low	low	low	moderate	moderate/high	moderate/high	moderate	very low
	Risk / Priority	moderate/high	low	low	low	moderate/high	moderate/high	moderate/high	low	very low

Table 5.11.3. List of selected diseases: fungus, protozoa and toxicosis. *Not clearly reported in the literature. ^Not sufficient data available.

	Disease	Fungus	Candida	Dermatophytosis	Cryptococcus	Protozoa	Neospora caninum	Leishmania	Toxicosis	Fluoroacetate (compound 1080)	Phalaris staggers	Pyrollizadine alkaloids	Sodium deficiency
	Species reported		Macropodoidea	Macropodoidea	Potoroos			Red kangaroos		All. WA animals tolerate high concentration	Macropodoidea	Macropodoidea	Macropodoidea
Known Presence	**Captive Pop's**		Yes	Yes	Yes		Unknown	Yes		Yes	*	*	*
	Wild Pop's		*	Unknown	Yes		Unknown	Unknown		Yes	*	*	*
	Transmission												
	Clinical signs				Respiratory signs and meningitis		Neurologiacal signs in dogs. Abortion in cattle						
	Lesions									No specific lesions detected at autopsy.			
	Comments		Identified only in captive animals	no reported in wild animals			no reported in wild animals	Identified near Darwin.A negative blood smear doesn't allow to rule out Leish.		Macropods (particularly PY) are susceptible. Unlikely: Woylie are resistant up to 100 mg/kg.	Isolated cases	Isolated cases	related to low concentration in soil and vegetation
	Reference		Jackson 2003	Speare *et al.* 1989	Vaughan *et al.* 2005, Vaughan pers com		Reichel 2000	Rose *et al.* 2004, Spratt 2005		King *et al.* 1981, Speare et al. 1989	Speare *et al.* 1989	Speare *et al.* 1989	Speare *et al.* 1989
Pathogenic Potential	**Likely Presence**		high	high	moderate		moderate/high	low		high	low	low	very low
	Independent		low	low	moderate		^	low		very low	low	low	very low
	In concert		low	low/moderate	moderate		^	moderate		low	very low	very low	very low
	Risk / Priority		low	low	low		low	low		very low	low	low	very low

5.11.7. References

Aguirre, A. A., G. M. Tabor, M. C. Pearl, R. S. Ostfeld, and C. House 2002. Conservation medicine: ecological health in practice. Oxford University Press, Oxford.

Armstrong, D., R. Jacob-Hoff, and U. S. Seal 2003. Animal movements and disease risk. IUCN/SSC, Apple Valley, MN.

Bodetti, T. J., K. Viggers, K. Warren, R. Swan, S. Conaghty, C. Sims, and P. Timms. 2003. Wide range of Chlamydiales types detected in native Australian mammals. Veterinary Microbiology 96:177-187.

Callinan, R. B., and B. Kefford. 1981. Mortalities associated with herpesvirus infection in captive macropods. Journal of Wildlife Diseases 17:311-317.

Daszak, P., and A. A. Cunningham. 1999. Extinction by infection. Trends in Ecology and Evolution 14:279.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging Infectious Diseases of Wildlife-Threats to Biodiversity and Human Health. Science 287:443-449.

Dickson, J., W. I. Hopkinson, and W. Coackley. 1980. Herpesvirus hepatitis in rat kangaroos. Australian Veterinary Journal 56:463-464.

Fowler, M. E., R. E. Miller, and Morris Animal Foundation. 2003. Zoo and wild animal medicine. W.B. Saunders, Phildelphia.

Haydon, D. T., M. K. Laurenson, and C. Sillero-Zubiri. 2002. Integrating Epidemiology into Population Viability Analysis: Managing the Risk Posed by Rabies and Canine Distemper to the Ethiopian Wolf. Conservation Biology 16:1372-1385.

Hooper, P. 1999. Kangaroo blindness and some other new viral diseases in Australia. Australian Veterinary Journal 77:514-515.

Hooper, P. T., R. A. Lunt, A. R. Gould, A. D. Hyatt, G. M. Russell, J. A. Kattenbelt, S. D. Blacksell, L. A. Reddacliff, P. D. Kirkland, R. J. Davis, P. J. Durham, A. L. Bishop, and J. Waddington. 1999. Epidemic of blindness in kangaroos--evidence of a viral aetiology. Australian Veterinary Journal 77:529-536.

Jacob-Hoff, R., M. Goold, and C. Reed. 2001. Translocation of brown teal from captivity to the wild: The application of a new process for developing quarantine and health screening protocols. Pages 231-235 in A. Martin, and L. Vogelnest, editors. Veterinary conservation biology; Wildlife health and management in Australasia. Proceedings of international Joint Conference of World Association of Wildlife Veterinarians, Wildlife Disease Association: Australasian Section. Australian Association of Veterinary Conservation Biologists and Wildlife Society of the new Zealand Veterinary Association, Australian Veterinary Association., Sydney.

Miller, P. 2003. Conservation breeding in IUCN/SSC, editor. Species. IUCN, Gland, Switzerland.

Munson, L., and W. Karesh. 2002. Disease monitoring for the conservation of terrestrial animals. Pages 95-102 in A. A. Aguirre, G. M. Tabor, M. C. Pearl, R. S. Ostfeld, and C. House, editors. Conservation medicine: ecological health in practice. Oxford University Press, Oxford.

Murphy, F. A., E. P. J. Gibbs, M. C. Horzinek, and M. J. Studdert 1999. Veterinary Virology. Academic Press, San Diego.

Old, J. M., and E. M. Deane. 2005. Antibodies to the Ross River Virus in Captive Marsupials in Urban Areas of Eastern New South Wales, Australia. Journal of Wildlife Diseases 41:611-614.

Reichel, M. P. 2000. *Neospora caninum* infections in Australia and New Zealand. Australian Veterinary Journal 78:258-261.

Rose, K., Kirkland, P., Claassens, R., Blumstein, D. and Cooper, D. 1999. An epizootic of sudden death in Tamar Wallabies (*Macropus eugenii*). Wildlife Diseases Association Australasian Section Annual Conference Proceedings p37.

Russell, R. C. 2002. Ross River virus: ecology and distribution. Annual Review Of Entomology 47:1-31.

Spalding, M. G., and D. J. Forrester. 1993. Disease monitoring of free-ranging and released wildlife. Journal of Zoo and Wildlife Medicine 24:271-280.

Spielman, D. 2001. The roles of contagious diseases in natural populations, endangered populations, captive populations, and in wildlife breeding, translocation and rehabilitation programmes. Pages 205-210 in A. Martin, and L. Vogelnest, editors. Veterinary Conservation Biology Wildlife Health and Management in Australasia. Proceedings of International Joint Conference of World Association of Wildlife Veterinarians, Wildlife Disease Association: Australasian Section, Australian Association of Veterinary Conservation Biologist of Wildlife Society of the New Zealand Veterinary Association, Australian Veterinary Association, Sydney.

Webber, C. E., and J. M. Whalley. 1978. Widespread occurrence in australian marsupials of neutralizing antibodies to herpesvirus from a parma wallaby. Australian Journal of Experimental Biology and Medical Science 56:351-357.

Wilks, C. R., B. Kefford, and R. B. Callinan. 1981. Herpesvirus as a cause of fatal disease in Australian wallabies. Journal of Comparative Pathology 91:461-465.

5.12. Disease synthesis

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Abstract

The Woylie Disease Reference Council (WDRC) has made a number of key contributions relating to diagnosing the decline in the woylie populations of southwestern Australia. These have included the identification of possible pathogenic organisms from woylies sampled from the Upper Warren and Karakamia Wildlife Sanctuary, the development of an extensive range of databases and reference materials, and furthering the knowledge of the health and diseases of this species as a whole. A summary of the disease investigations thus far is presented, and suggestions made as to what further investigations are recommended.

5.12.1. Introduction

The Woylie Disease Reference Council has made a number of valuable contributions and findings through investigations of diseases of potential concern in population declines of woylies in southwestern Australia. Results from individual and collaborative investigations have contributed to a clearer understanding of the health status of woylies, including the establishment of a disease risk assessment table, development of a haematological reference range, refinement of clinical skills in managing sick and injured woylies, and initial establishment of a pathologic reference library of disease processes found in woylies. A number of potentially pathogenic organisms have been identified in woylies sampled from the Upper Warren and Karakamia Wildlife Sanctuary, including haemoparasites (species of Trypanosomes and Piroplasms), *Toxoplasma*, ectoparasites and gastrointestinal protozoan and helminth parasites. The findings of investigations into these infectious agents will be summarised below in an attempt to pull this information together. We will also highlight where information is currently deficient, and provide recommendations for prioritising further investigations.

5.12.2. Summary of Disease Investigations

A number of potentially pathogenic organisms have been identified in woylies from the Upper Warren and Karakamia populations in Western Australia. From investigations undertaken to date, there is insufficient evidence to indicate any of these pathogens as a primary infectious agent responsible for these declines. This is largely due to the absence of evidence rather than there being evidence that does not support disease as a potential factor. Most animals (>90%) trapped in the field or submitted for necropsy were found to be in good body condition, suggesting an acute process is involved in the loss of animals. This is further supported by the rate of the decline that has been observed. Demographic and survival information suggests the declines are driven primarily by adult mortality rather than reduced fecundity, and therefore diseases affecting reproductive success appear less likely to be of concern in this situation. Findings from clinical and necropsy examinations have not identified a particular disease as a common cause of death to date. However, of the pathogens currently investigated, the evidence available is suggesting that *Toxoplasma* and *Trypanosoma* may have contributed, either directly or indirectly, to declines in the Upper Warren population. Other, yet to be identified stress factors and/or infectious agents have also been suggested for future investigation and will be prioritised in the discussion below.

Toxoplasma is a systemic protozoan parasite known to particularly affect Australian marsupials (Section 5.7 *Toxoplasma*). Investigations have shown a low seroprevalence to *Toxoplasma* infection of 5.8% in Upper Warren populations compared to a zero prevalence in Karakamia and Dryandra. This is difficult to interpret in itself but may indicate the presence of subclinical or clinical disease in Upper Warren populations. Sequential sampling revealed a decrease in seroprevalence over time. There are various explanations for this, including the possibility that seropositive animals have died, perhaps directly or indirectly as a result of Toxoplasmosis, and further data analysis is recommended. It is possible that other pathogens (such as *Trypanosoma*) may predispose the woylie population in the Upper Warren to the pathogenic consequences of infection with *Toxoplasma*. The lack of pathological evidence of Toxoplasmosis in necropsy specimens, and the lack of knowledge of the disease processes associated with these parasitic infections in natural wild populations of marsupials, precludes any definitive conclusions at this stage. Further investigations should include examination of currently available data to assess for evidence of co-infection of these organisms and the potential subclinical effects, as well as to assess changes to serological titres within an individual over time. Evidence for the congenital transmission of *Toxoplasma* was also found, which may be significant if further research indicates that this mode of transmission is common and could serve to maintain *Toxoplasma* in wild populations without the involvement of the definitive host, the cat.

Trypanosomes are a protozoan blood parasite that can result in chronic illness with weight loss, anaemia and death. They are primarily spread via an arthropod vector. Investigations of Trypanosome infections in woylies indicated a relatively high prevalence of infection in the Upper Warren population, as compared to the Karakamia population. Further investigation, using molecular tools will be undertaken (discussed below). Investigations into the potential subclinical effects of this infection and demographic variations on prevalence using existing data are recommended.

Gastrointestinal parasite infections were more prevalent in Upper Warren than in the Karakamia woylie population, which is interesting given the higher density of woylies in Karakamia compared to Upper Warren. Preliminary data analysis has found no evidence of a relationship between infection and woylie condition. It is possible that the differences in parasitism between populations may have important indirect effects on host mortality. Further analysis of existing data to look at variations in prevalence on a demographic basis, and to investigate possible subclinical effects of infection, is recommended.

A variety of ectoparasites have been identified but their role in the decline is uncertain. Ectoparasites can, in significant numbers, be primary pathogens and result in symptoms such as anaemia. Perhaps more importantly, they can act as vectors for haemoparasites, for example Trypanosomes and *Rickettsia*. Identification of ectoparasite samples will continue, along with analysis of existing data to assess effects on fecundity and host survival. Investigations into the role of ectoparasites as vectors have also been proposed and are discussed below.

Evidence of infection with *Salmonella*, and the potential for woylies to be infected with *Rickettsia* has been found in woylies from the Upper Warren region; however the significance of these findings is likely to be low in relation to these population declines.

Thirty one woylies were submitted for necropsy. In 15 cases a cause of death could not be determined by gross or microscopic examination however specimens from cadavers were often limited due to predation/scavenging. A number of potential diseases, discussed in section 5.11, are yet to be investigated. A number of viruses have been reported from macropods in Australia, some potentially resulting in acute death (e.g. Macropod Herpesvirus). To date, investigation into viral disease in woylies has been limited and are discussed further below.

The role of toxic exposure in the population decline has not been investigated to date. Toxins found in native plants such as fluoroacetate (1080) and cardiac glycosides are possible agents, although the LD_{50} of fluoroacetate is significantly higher in woylies than for all domesticated mammals. Further investigation of the role of poisonous plants in the decline of the woylie could include examination of the gastro-intestinal contents to assess for exposure to toxic plants.

5.12.3. Future priorities

A range of areas for future research have been suggested from the previous discussions and relevant sections from Chapter 5. These will be discussed further below and recommendations made as to which are considered a priority.

Viruses

Current data is deficient with respect to potential viral diseases. Those reported to have occurred in macropods are discussed in Chapter 5 (Section 5.11 Disease and risk assessment). In particular, macropod herpesvirus and macropod orbivirus have previously resulted in disease epidemics in captive and wild macropod populations (Section 5.11). It is recommended that a priority of future research is a focus on key viral pathogens as identified in Section 5.11.

Trypanosomes

A PCR was recently developed at Murdoch University veterinary parasitological department to assay for the presence of *Trypanosoma* spp. in the blood samples of woylies. This complemented the current microscopic survey of blood smears. Blood kept in archives and future samples will be assayed by PCR for the presence of *Trypanosoma* spp.

Transfection Study

A project has been designed to monitor the effect of trypanosomes in endemic populations of woylies and naïve populations. The study will focus on methods of transmission of trypanosomes and the effect on the health of woylies.

Arthropod vectors

Murdoch University and Department of Environment and Conservation will jointly support either a summer school project or honours project to collect and classify insects from the Upper Warren area that could be potential vectors for the haemoparasites or arboviruses.

Epidemiological and ecological data analysis

More detailed analysis of parasite prevalence in relation to multiple infections/co-occurrence, different species of co-habiting host (where possible), geographic location, host demography, host condition, clinical signs of disease in the host and environmental factors operating on the host is recommended (discussed earlier in this chapter). This analysis will use the established large database and will provide valuable epidemiological information regarding these potential pathogens.

Biochemistry

Serum biochemistry provides a broad approach to disease investigations, allowing investigation of organ disease and providing some focus for future investigations. Little information is available on serum biochemistry of the woylie and such data, using serum banks already available, would complement current and future pathogen data and improve identification of the aetiological agents of disease.

5.12.4. Conclusion

A number of potential pathogenic organisms have been identified and investigated through various projects represented by the WDRC, helping to further understand various health and disease aspects of the woylie. From the analyses undertaken so far, a primary pathogenic agent has not been identified that may result in population declines observed in woylies in southwestern Australia. Preliminary data indicates a possible role for *Toxoplasma* and Trypanosomes in declining populations, however this information should be interpreted with caution and further analysis is recommended to investigate these pathogens in more detail. A number of key viral pathogens that could result in the decline patterns observed have not been investigated to date, and it is recommended that studies into these diseases be prioritised in the near future. A number of other research focuses have also been presented and discussed. Other outcomes from the work of the WDRC include establishing reference health information, initial development of a pathologic reference library of woylie diseases, and further development of clinical skills both in the field and in a hospital setting

5.12.2. References

Maxie G. 2007. "The Haematopoietic system" in the "Pathology of domestic animals" Elsevier Saunders

CHAPTER 6 CONSERVATION GENETICS

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Abstract

A crucial component of the Woylie Conservation and Research Project (WCRP) is a sound understanding of the genetics of this species.

Our aims were to characterise woylie genetics and use this information to focus on outcomes that will be directly relevant to conservation and recovery of the species and to incorporate these findings into the demographic management of declining populations.

6.1.1. Introduction

The role of genetics as an important factor influencing population dynamics has been analysed on both: theoretical and empirical grounds (Frankham, 1995). The recent development of the study of microsatellite loci for population genetics offers the opportunity to use non invasive sampling techniques and, at the same time, to have a good understanding of the genetic dynamics of wild populations (Goldstein and Schlèotterer, 1999; Sunnucks, 2000). The species appears to have undergone two recent (genetic) bottlenecks. One occurred following European settlement, and another as recently as four years ago. A possible outcome of this well-known genetic phenomenon would be a decreased capacity of the population to cope with changes as a result of the loss of variability and this may ultimately impact on the long-term survivorship of the population. As such, this issue should be considered an important aspect in the investigation of the recent woylie decline.

6.1.2. Methods

We used mtDNA and microsatellite loci to achieve the following aims:

- Characterise the historical genetic profile of the woylie species across its former range since the settlement of Australia by Europeans using ancient DNA approaches.
- Characterise the genetics of remaining contemporary extant woylie populations in Western Australia (i.e. Upper Warren, Dryandra and Tutanning), include quantifying to the levels of genetic variability, estimating the effective population size within and among extant remnant.
- Investigate the genetic consequences of previous population declines and range contractions by comparing available historical material with contemporary material from remaining indigenous populations (see above).
- Model and quantify the genetic responses within translocated populations in Western Australia and South Australia based on the characteristics of the founding cohort (e.g. size, genetic diversity, etc), the characteristics of the translocation population establishment (e.g. rate of increase, population size, density, time since establishment, etc) and the effect of post establishment animal supplementation (e.g. introduction of additional animals post-establishment for the purposes of increasing genetic diversity). Genetic responses include correlating and assessing the reproductive success of translocated animals; estimating the effective size of the founding population; and, quantifying the extent of loss of genetic diversity.
- Based on the findings of the above objectives, predict the genetic implications of recent woylie declines, and the likely consequences for future species recovery and

conservation. This may allow specific populations to be identified as high priority for conservation efforts.

- Investigate whether there are any relationships between woylie population declines (and associated demographic change such as survivorship, reproductive success, etc) and the genetic attributes of affected and unaffected populations (i.e. a complimentary component of the existing population comparison study).
- Describe information on paternity, social structure, home range and individuals movements between adjacent populations.

Tissue samples have been collected from animals trapped at the Population Comparison Study (PCS) sites, and Western Australian populations (Dryandra, Tutanning and Batalling) as well as populations that were reintroduced to South Australia. In addition, tissue samples collected during (previous) translocations have been made available for this study by other researchers. Using this material, we optimised a range of microsatellite primers that were developed from studies of other macropods and potoroos. These primers were tested to verify their suitability for investigating the molecular ecology of the woylie.

6.1.3. Results

To date, more than 1200 tissue samples have been collected, collated, incorporated into a sample database. All samples are stored in the wildlife genetic collection at Murdoch University (Table 6.1.1).

Table 6.1.1. Sample collection field sites detailing the number (N) of tissue samples collected for use in this study.

Sampling locality	Number of samples (N)
Upper Warren	535
Karakamia Wildlife Sanctuary	256
Batalling State Forest	89
Dryandra Nature Reserve	60
Tutanning Nature Reserve	44
St Peter's Island, SA	119
Venus Bay Island A, SA	31
Venus Bay Peninsula Conservation Park, SA	50
Wedge Island, SA	80
Total	**1264**

DNA extractions were carried out on a subset of more than 250 of these samples. Twenty-eight primers that were originally developed for different Macropodidae species were tested to assess their suitability and polymorphism in woylies (Table 6.1.2). Nineteen of those produced an amplified PCR product when tested with woylie DNA. Of those, 13 produced reproducible and variable information. For a small test-sample (N=8), we recorded heterozygosity values of between 70 and 86% (mean heterozygosity = 79 ± 5.7%). All 13 loci were highly polymorphic, with between four and 12 alleles at any locus and the average of number of alleles per locus of 6.69 (S.D. = ± 2.05;) (Table 6.1.3).

Table 6.1.2. Information available related to the primers described from a range of Macropodidae species that were tested for polymorphism in samples from the woylie. Readability is how easily the DNA profiles could be interpreted and indicated as a score from 1 (very low) to 5 (very good).

Locus	Gene Bank	Size range	Readability	Reference
Bt64			4	(Pope *et al.*, 2000)
Bt76			5	(Pope *et al*.., 2000)
Bt80			5	(Pope *et al.*, 2000)
Pl2	Y09050	150-160	1	(Luikart *et al.*, 1997)
Pl3	Y09051	141-159		(Luikart *et al.*, 1997)
Pl13	Y09052	77-113		(Luikart *et al.*, 1997)
Pl18	Y09053	126-134		(Luikart *et al.*, 1997)
Pl22	Y09054	124-156		(Luikart *et al.*, 1997)
Pl26	Y09055	164-184	4	(Luikart *et al.*, 1997)
Y175			4	(Zenger *et al.*, 2002)
Y170				(Pope *et al.*, 1996)
Y151			4	(Pope *et al.*, 1996)
Y76			1	(Pope *et al.*, 1996)
Pa597	U30636		3	(Spencer *et al.*, 1995)
Pa593	U30633		5	(Spencer *et al.*, 1995)
Pa297	U30634		2	(Spencer *et al.*, 1995)
Pa385	U30632		3	(Spencer *et al.*, 1995)
Pa595	U30635			(Spencer *et al.*, 1995)
B90				(Pope *et al.*, 2000)
B123				(Zenger *et al.*, 2002)
G31-1	AF322629	118-136		(Zenger and Cooper, 2001b)
Me15	AF025909	225-270		(Taylor and Cooper, 1998)
Me16	AF025910	240-280		(Taylor and Cooper, 1998)
Me17	AF025911	110-140		(Taylor and Cooper, 1998)
T17-2	AF326948	115-147	4	(Zenger and Cooper, 2001a)
T31-1	AF326953	115-137		(Zenger and Cooper, 2001a)
MeY01	DQ641481	340-344		(Macdonald *et al.*, 2006)
MeY37	DQ641488	179-181		(Macdonald *et al.*, 2006)

Table 6.1.3. Locus characteristics: preliminary results.

Locus	*N*	H_O	H_E	N_A	N_E
Bt76	14	1.000	0.857	9	7.00
Bt80	16	0.875	0.820	7	5.57
T172	16	0.500	0.773	5	4.41
Pl2	16	1.000	0.797	6	4.92
Bt64	14	0.571	0.704	7	3.38
Pa297	10	0.400	0.800	6	5.00
Pa597	16	0.000	0.719	4	3.56
Pa595	14	0.286	0.816	6	5.44
Pl26	16	0.625	0.695	4	3.28
Y151	16	0.750	0.898	12	8.00
Y170	16	0.875	0.789	6	4.74
Y175	16	0.875	0.844	8	6.40
Pa593	16	0.875	0.773	7	4.41
Mean	**15.1**	**0.664 ± 0.291**	**0.791 ± 0.057**	**6.69 ± 2.05**	**4.84 ± 1.10**

H_0 Observed heterozygosity.; H_E Expected heterozygosity (Nei 1973). ; N_A Number of alleles.; N_E Number of expected alleles.

6.1.4. Discussion

We have made significant progress towards achieving the development of a set of polymorphic microsatellite markers for the study of the molecular ecology of the woylie. Once these informative primers have been further refined and optimised we will be able to generate a rapid amount of information to interpret any underlying patterns of genetic contributions in the recent demographic changes that appear to have occurred in populations of the species.

The reliable interpretation of the PCR-amplification products is extremely good for eight primers (Bt 64, Bt 76, Bt 80, Pl 26, Pa 593, T17-2, Y151, and Y175) while the remaining (Pl 2, Pa 385, Pa 597, Pa 297 and Y76) are yet to be fully optimised. The remaining 15 primers were either difficult to interpret, monomorphic or did not produce a PCR product.

Based on the allele frequencies, we found from our preliminary screening that the average Hardy-Weinberg expected heterozygosity (in the small number of woylies tested) to be very high (79%; see Table 6.1.3 for details; Nei, 1973). This level of heterozygosity is similar to the amount of variability found in other marsupial species (Pope *et al.*, 2000, Eldridge *et al.*, 2004). In addition, this result suggests that the probability of obtaining identical DNA profiles from two randomly chosen woylies would be more than one in 100 million. As a result, the DNA profiles are unique to a each individual and consequently great confidence can be placed in correctly identifying individuals and their relationship to other woylies.

6.1.5. Future directions

We are planning to optimise the polymorphic primers in October. Once an efficient set of conditions has been achieved, most of the remaining genetic data should be generated by the end of this year. It is anticipated that the genetic analysis should be concluded in early 2008.

6.1.6. Conclusion

We believe that this investigation represents an interesting case study, which provides not only important information directly relevant to woylie management and conservation but also a model for other species that share similar management history and ecology. We are confident that the number of primers that we have been able to use for this genetic study is appropriate to answer our original aims. A large robust set of primers now exist to answer these questions. Coupled with samples obtained from such a large number of locations and a large number of samples from within each, this should allows to work with reasonable statistical confidence.

6.1.7. Acknowledgments

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6.1.8. References

Eldridge M.D.B., Kinnear J.E., Zenger K.R., McKenzie L.M. and Spencer P.B.S. (2004) Genetic Diversity in Remnant Mainland and mainland and "Pristine" Island Populations of Three Endemic Australian Macropodids (Marsupialia): Macropus Eugenii, Lagorchestes Hirsutus and Petrogale Lateralis. Conservation Genetics, 5, 325-338.

Frankham R. (1995) Inbreeding and Extinction: A Threshold Effect. Conservation Biology, 9, 792-799.

Goldstein D.B. and Schlèotterer C. (1999) Microsatellites: evolution and applications, Oxford, Oxford University Press.

Luikart G., Painter J., Crozier R.H., Westerman M. and Sherwin W.B. (1997) Characterization of microsatellite loci in the endangered long-footed potoroo Potorous longipes. Molecular Ecology, 6, 497-498.

Macdonald A.J., Sankovic N., Sarre S.D., Fitzsimmons N.N., Wakefield M.J., Graves J.A.M. and Zenger K.R. (2006) Y chromosome microsatellite markers identified from the tammar wallaby (Macropus eugenii) and their amplification in three other macropod species. Molecular Ecology Notes, 6, 1202-1204.

Nei M. (1973) Analysis of gene diversity in subdiveded populations. Proceedings of the National Academy of Sciences of the United States of America, 70, 3321-3323.

Pope L.C., Estoup A. and Moritz C. (2000) Phylogeography and population structure of an ecotonal marsupial, Bettongia tropica, determined using mtDNA and microsatellites. Molecular Ecology, 9, 2041-2053.

Pope L.C., Sharp A. and Moritz C. (1996) Population structure of the yellow-footed rock-wallaby Petrogale xanthopus (Gray, 1854) inferred from mtDNA sequences and microsatellite loci. Mol Ecol, 5, 629-40.

Spencer P.B., Odorico D.M., Jones S.J., Marsh H.D. and Miller D.J. (1995) Highly variable microsatellites in isolated colonies of the rock-wallaby (Petrogale assimilis). Mol Ecol, 4, 523-5.

Sunnucks P. (2000) Efficient genetic markers for population biology. Trends in Ecology and Evolution, 15, 199-203.

Taylor A.C. and Cooper D.W. (1998) A set of tammar wallaby (Macropus eugenii) microsatellites tested for genetic linkage. Molecular Ecology, 7, 925-926.

Zenger K.R. and Cooper D.W. (2001a) Characterization of 14 macropod microsatellite genetic markers. Animal Genetics, 32, 166-167.

Zenger K.R. and Cooper D.W. (2001b) A set of highly polymorphic microsatellite markers developed for the eastern grey kangaroo (Macropus giganteus). Molecular Ecology Notes, 1, 98-100.

Zenger K.R., McKenzie L.M. and Cooper D.W. (2002) The First Comprehensive Genetic Linkage Map of a Marsupial: The Tammar Wallaby (Macropus eugenii). Genetics, 162, 321-330.

CHAPTER 7 SUMMARY OF PROGRESS AND INTERIM FINDINGS

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Abstract

The responses to the report of woylie declines in the Upper Warren included an initial situation assessment of woylie populations throughout the southwest, an early-response workshop, the establishment of the Woylie Conservation Steering Group and the development of the Woylie Conservation Research Project (WCRP). Using a decline diagnosis framework broadly based on the 'declining-population paradigm, the WCRP consists of three major components; I) Upper Warren fauna monitoring that built on, enhanced and co-ordinated previously independent existing activities, II) Meta-analysis of existing datasets that were aggregated into a single database, and III) a population comparison study (PCS) designed to discriminate factors and attributes associated with contemporary declines. The PCS has five main lines of enquiry; a) woylie density and demographics, b) woylie survival and mortality, and investigations into the key putative agents of decline, c) predators, d) resources, and e) disease.

Although it remains premature to identify the possible cause(s) of the woylie declines, the preliminary results can provide some early hints regarding what ongoing work is likely to suggest might be the likely agents of decline. Factors that are not probably the primary agents of decline include habitat loss / modification, fire, direct human interference from trapping and resources including food. Climate may be associated with woylie declines at Venus Bay Peninsula (VBP), South Australia and cannot be ruled out as a factor in Western Australia, but seems unlikely for the Upper Warren populations at least. Given the lack of fox activity or density monitoring data associated with most of the observed woylie declines it is not possible to determine whether they may be a major agent of decline. This is, however, considered unlikely for the Upper Warren region given that during the WCRP, foxes only accounted for 15% of the implicated primary predators/scavengers associated with observed mortalities and none of the mortalities at the Balban PCS site (which underwent a >80% decline in 12 months) were attributed to foxes.

The rapid and substantial woylie declines are driven at least by increased adult mortality. Emigration of animals elsewhere is not supported by the evidence. Whether recruitment into the adult (breeding) population is involved in the declines can not yet be established, however, the preliminary exploration of the data suggests breeding rates (prevalence of pouch-young) are not associated with the declines.

The leading speculative hypothesis (i.e. untested) for the cause(s) of the declines is presented. In summary, multiple interactive factors are expected responsible, with disease considered the most likely primary and ultimate agent of decline. The symptoms and unequivocal confirmation of the disease and the related interacting factors remain elusive at this stage, although some key suspects have been identified including *Toxoplasma*, *Trypanosoma sp. nov.*, possible synergistic effects between the two parasites and the involvement of stressors that may trigger the disease. Other infective agents may also be involved. As a consequence of the disease(s), opportunistic and exploitative predation/scavenging, predominantly by cats, are likely the most proximately-related factor associated with the deaths of the woylies. Whether the predation/scavenging occurs on moribund or dead animals that would die regardless, or whether in the absence of predation in general, the woylies would otherwise recover and survive remains unknown.

7.1. Summary of progress

In response to the initial report (26 October 2005) of a suspected woylie decline at a site in the Upper Warren, a preliminary investigation of available data and additional field surveys were conducted between November 2005 and February 2006 to assess the situation. This work was led by Science Division in close collaboration with Nature Conservation personnel in Manjimup. In February 2006 a three-day workshop on the 'Recent Mammal Declines in the Southwest' was convened at Perup Ecology centre and attended by 32 Departmental personnel directly associated with woylie and fauna monitoring, research and/or conservation and management. The objectives of the workshop were;

1. Provide an overview and understanding of the recent mammal declines in the southwest
2. Examine the potential cause(s) of these recent declines and associated evidence
3. Identify improvements to mammal monitoring protocols for the Upper Warren (a model for Corporate-level improvements via *Western Shield*)
4. Follow-up task force meeting to determine future directions (priorities, strategies, plans, organisational structure, improved mammal monitoring protocols, actions, budget, and links with other corporate working groups)

Among the outcomes of the workshop was the transformation of the initial response task force into the 'Woylie Conservation Steering Group' with broader Departmental representation. Response priorities included the development of a research and monitoring program proposal to investigate the causes of the woylie declines (i.e. WCRP), invoke a moratorium on the movement and translocation of woylies, inform and instruct all Districts/Regions and researchers associated with woylie populations, complete a woylie conservation status review, establish a 'monitoring protocols review panel' that would report to the *Western Shield* Operations Research Committee (WSORC) and initiate collaborations with existing mesopredator research programs.

A situation report and WCRP proposal was presented to the Corporate Executive in May 2006 and the project commenced in July 2006 having received support from the Director General and 12–months funding via the 'Saving Our Species' (Biodiversity Conservation Initiative) program.

The WCRP has been addressing the first five steps of the woylie decline diagnosis framework during 2006 and 2007 (*sensu lato* declining-population paradigm, Caughley, 1994).

Woylie decline diagnosis framework

1. Is the decline real?
2. Decline characteristics
3. Understand the species' ecology
4. Identify all potential causes
5. Shortlist using circumstantial evidence
6. Direct evidence –test putative causes
7. Conservation and management responses (experimental)

The WCRP has three major components that together, address the first five steps of the above diagnosis framework.

1) <u>Upper Warren Fauna Monitoring</u> – an enhancement and co-ordination of existing monitoring and research activities – that provides;

a) six-monthly information up-dates on population change and associated characteristics at the regional scale and,
b) a regional-scale means of collecting data on woylies and putative agents of decline to complement the finer-scaled population comparison study.

2) Meta-analysis of existing data sets (aggregated into a single relational database providing data on 25,479 woylie captures over 33 years) to;

a) Confirm that the declines are real

b) Quantify the spatial, temporal and demographic characteristics of the woylie decline, which in turn will,

c) Provide circumstantial evidence that will aid in the identification of the possible causes of decline.

3) Population Comparison Study (PCS) is a detailed investigation of woylies and the possible agents of decline. This principally involves six sites that support populations at different stages of decline;

- Declined populations now at low densities: Boyicup and Winnejup (Upper Warren Region)
- The last remaining moderate-density woylie populations in the Upper Warren region: Keninup, Warrup and Balban
- High-density and stable population - Karakamia Wildlife Sanctuary (50 km east of Perth), a fenced (i.e. closed) population

The five main lines of enquiry to be investigated at the population comparison study sites are;

a) Woylie density and demographics

b) Woylie survival and mortality

c) Predators

d) Resources

e) Disease

Field work was largely completed by August 2007. Data and information management and the commencement of preliminary analyses have ensued.

7.2. Summary of key findings

7.2.1. Preliminary inferences (i.e. early hints) regarding possible causes of recent woylie declines

Extreme caution is required when inferring from the premature results and analyses generated to date. It must be emphasized that all results presented in this report are preliminary and require substantial development and verification before any reasonable levels of confidence can be attributed to them. Having considered this, and within the context of an urgency to appropriately respond to the rapidly changing situation with respect to the woylie declines, it is possible to draw some early hints from the balance of associative evidence collected and processed to date. The use of these early hints *must* be regarded in this context and any responses made on these hints alone have the potential to jeopardize the recovery and long-term conservation of the woylie (e.g. the conservation efforts for the California Condor inadvertently facilitated the species extinction in the wild in the 1980's; Caughley 1994). The intent of this summary is therefore strictly to provide managers and collaborators with a coarse-resolution anticipation of what subsequent and more complete analyses may well indicate as being *some* of the *primary* agents of recent woylie declines.

7.2.2. Comparison of postulated responses with available evidence

Table 7.2.1 summarizes the result of the application of logic in an analysis of the data available for, and relevant to, determining the hierarchy of causes of the decline of the woylie. A truth table or logic matrix assists in the ordering of the research data acquired, reducing the risk of missing potentially relevant factor. It can also aid in the selection of variables worth measuring. This table helps communicate some of the complexity and nuances associated with this investigation. As with any conceptual model, the truth table is a means to an end, and is not the end in itself.

Models simplify reality and are not intended to accommodate all of the available information. Assumptions and limitations of this truth table include; i) primary factors are initially considered in isolation when multiple interacting factors are more likely responsible; ii) focus on linear/mechanistic treatment of potentially non-linear/biological relationships; iii) generalisation and restriction of response behaviours to only the potentially most prominent (i.e. limited capacity to consider secondary relationships); and, iv) limited capacity to discriminate between cause and effect.

The truth table, being a condensation of many facts, does not distinguish quality of evidence, the full diversity and variability of the evidence or the inferential power of the evidence (e.g. the result of no change/difference may be due to evidence limitations, i.e. potentially falsely summarised). While relative weighting and other adjustments are possible, the complexity of the model and the necessary associated assumptions substantially escalate when doing so, with potentially greater risk of becoming a less realistic model.

Expert judgement was used to limit the most likely variable behaviours under the key putative agents of decline and the preliminary evidence results – based on available evidence that may or may not have been fully communicated within this report. When the preliminary evidence is subsequently compared with the postulated behaviour of the selected variables, eight key discriminating factors emerged. On this basis, the results of this exercise suggest that the weight of available evidence supports disease as the most likely principal factor associated with the woylie declines in the Upper Warren region.

The most valuable outcome of the truth table is likely to be the stimulation that it offers for further discussion and development of the research agenda, consideration of the evidence, and progress towards identifying the chain of cause involved in recent woylie declines.

Table 7.2.1. Truth table (logic matrix) of postulated direct causal relationships of selected variables (table rows), assuming a single primary cause for recent woylie declines (columns 2-5).

Initially prepared in February 2006, the postulated responses are compared with the preliminary results from the WCRP in the Upper Warren region.
Warning: this table needs to be interpreted within the context intended (see text).
Note: the key discriminating variables are highlighted

General indicators	Live trapping	Food Resources	Predators	Disease	Initial evidence
POPULATION MECHANICS					
Immigration	↔ / ↑	↔ / ↓	↔ / ↓	↔ / ↓	↔
Recruitment	↔ / ↓	↔ / ↓	↓	↔ / ↓	↔
Emigration	↔	↑	↔ / ↑	↔	↔
Mortality	↔ / ↑	↔ / ↑	↑	↔ / ↑	↑
WOYLIE CAGE TRAPPING					
Trap shyness – learned avoidance	↑	↔ / ↓	↔ / ↑	↔	↔
Trapping intensity / frequency	↑	↔	↔	↔ / ↑	↔ / ↓
WOYLIE DEMOGRAPHICS					
Risk with increasing woylie density	↔ / ↓	↑	↓	↑	↑
Animal condition	↔ / ↓	↓	↔ / ↑	↔ / ↓	↔ / ↑
% Adult Females breeding	↔ / ↓	↔ / ↓	↔ / ↑	↔ / ↓	↔
Age structure (SA: Adult ratio)	↓ / ↑	↓ / ↑	↓ / ↑	↔ / ↓ / ↑	↔
Male: Female Ratio by age class	↓ / ↑	↓ / ↑	↔ / ↓ / ↑	↔ / ↓ / ↑	↔
Population turnover (% New adults)	↑	↔ / ↑	↔ / ↑	↔ / ↑	
Animal longevity	↔ / ↓	↔ / ↓	↔ / ↓	↔ / ↓	
OTHER SPECIES					
Competitor abundances	↔	↓ / ↑	↔ / ↑	↔ / ↓ / ↑	↔ / ↓ / ↑
Analogous prey species	↔	↔	↓	↔	↔ / ↓
Taxonomically-related species	↔	↔ / ↓	↔ / ↓	↔ / ↓	↔
Ecologically-related species	↔	↔ / ↓	↔	↔ / ↓	↔ / ↓
Fox activity/abundance	↔	↔ / ↓	↔ / ↑	↔ / ↑	↔
Cat activity/abundance	↔	↔ / ↓	↔ / ↑	↔ / ↑	↑
Chuditch abundance	↔	↔ / ↓	↔ / ↑	↔ / ↑	↔
Raptor abundance	↔	↔ / ↓	↔ / ↑	↔ / ↑	↔ / ↑
Fox control effort/regime changes	↔	↔	↔ / ↑	↔	↔
Fox control timing relative to heavy rain	↔	↔	↔ / ↑	↔	↔
WOYLIE RESOURCES					
Food	↔	↓	↔	↔	↔
Diet	↔	↓	↔	↔	↔
Shelter	↔	↓	↔	↔	↔
Vegetation change/differences	↔	↔ / ↑	↔ / ↑	↔	↔
LANDSCAPE PROCESSES					
Weather pattern changes	↔	↔ / ↑	↔	↔ / ↑	↔
Extreme weather events	↔	↔ / ↑	↔	↔ / ↑	↔
Changed fire regimes	↔	↔ / ↑	↔ / ↑	↔	↔
Jarrah Dieback prevalence	↔	↔ / ↑	↔ / ↑	↔	↔
Logging / other mechanical disturbance	↔	↔ / ↓ / ↑	↔ / ↑	↔ / ↑	↔
Habitat loss (e.g. landclearing)	↔	↔ / ↑	↔ / ↑	↔ / ↑	↔
WOYLIE HEALTH					
Clinical signs of disease	↔	↔	↔	↔ / ↑	↔
Pathological evidence of disease	↔	↔	↔	↔ / ↑	↔
Incidence of field health reports	↔ / ↑	↔ / ↑	↔	↔ / ↑	↑
Tally of key discriminating evidence	5	3	6	8	

7.2.3. Not primary agents of decline

Based on the available evidence, the following factors are considered in all likelihood not to be primary factors causing decline either in isolation or in association with other factors;

- Habitat loss / modification (including logging)
- Fire (prescribed burning or wildfires)
- Direct human interference (live-trapping)
- Resources

7.2.3.1. Why resources are not considered as a primary agent of woylie decline

Resources may potentially limit and/or cause faunal declines due to changes in the abundance and/or availability of resources. Changes in resource abundance may result from environmental change, management practices (e.g. fire, logging, etc), introductions (e.g. weeds, diseases such as *Phytophthora cinnamomi*), and/or over exploitation. Changes in resource availability or access primarily result from competition.

Of the resources needed for the persistence of fauna populations, food is considered the most likely limiting resource that may be a putative agent of the woylie declines. Water is not considered to be an issue given woylies reportedly source all of their requirements directly from their food. Shelter is unlikely to be a limiting resource given woylies build their own nests in low understorey vegetation and there have been no apparent substantial structural or floristic differences in the vegetation associated with the declines. Space is not considered limiting given there has not been a reduction in native vegetation cover on the DEC-managed lands in the Upper Warren region, Dryandra, Batalling, etc and changing densities of other fauna species cannot satisfactorily account for the woylie declines in this manner. Access to reproductive mates are not considered a primary factor in the decline of the woylie given the pre-decline densities, however, this may well be a limiting factor for the recovery of the species.

Preliminary evidence from the population comparison study provides several lines of evidence consistent with food resource limitations not being associated with woylie declines. This includes;

Evidence from Karakamia

- History of population establishment (1995) is temporally comparable to the recovery and/or establishment of other wild woylie populations and continues to support a very high density of woylies in the absence of predators (~2 / ha).
- The average weight and size of woylies has reduced at Karakamia since the population was established, i.e. in association with the density of woylies increasing and stabilizing. Karakamia woylies are now significantly lighter than woylies at Upper Warren and elsewhere - this reduction/difference in weight/size has been interpreted as a response, in part at least, to food resource limitation, having reached carrying capacity.
- Woylies have a seasonal breeding pattern with little or no breeding occurring over the summer months, in contrast to other woylie populations that display continuous breeding – this has been interpreted as a response to seasonal food resource limitation.

Evidence from Upper Warren

- Adult male condition (biometric function of size/weight relationship) remained stable in association with the population decline at Camelar. Adult female condition increased in association with the population decline at Camelar (Wayne 2006 – Mammal decline workshop). This is inferred as indicating that the population was not resource-limited during the declines.
- Although there were site (spatial) differences in the biometric condition of woylies, the meta-analysis found no relationship with population decline.

Evidence from the woylie diet and food resources

- No apparent significant vegetation structure or floristic changes associated with declines or between population comparison sites (substantially more analyses required to verify this). This includes no apparent gross changes in *Phytophthora cinnamomi* (jarrah dieback) infections at the PCS sites or in association with declines.
- Hypogeal fungi are considered a primary (but not exclusive) dietary component. The harvesting of the fruiting bodies by woylies and other animals are considered extremely unlikely to negatively-affect subsequent fruiting. Rather quite the opposite is expected, with fauna such as the woylie being necessary for the dispersal of hypogeal fungi throughout the environment.
- Preliminary data from the dietary analysis of woylies using scats indicates that there are no substantial spatial differences in the fungi consumed between Upper Warren PCS sites, however, there are substantial temporal differences (common across Upper Warren PCS sites) in the species composition.

Despite food resources not being considered at this stage as a primary agent of decline this does not negate the possibility of food resources being involved in some way. Furthermore, being able to unequivocally disregard food resources as a putative agent of decline is as important as establishing cases for other key putative agents of decline. Irrespective of the role of food resources in the decline of woylies, a solid understanding of this component of woylie ecology may play an important role in managing the recovery of the species. For these and other reasons, it remains important to continue the current PhD research into the food resources of woylies in relation to the recent woylie declines.

7.2.4. Possible primary agents of decline

7.2.4.1. Climate

Notwithstanding the inherent complexity, climate is possible but not likely to be a direct or primary agent of woylie declines in the Upper Warren, however, this remains to be more satisfactorily verified. There is some weak associative evidence that climate may be associated to woylie declines in the drier, inland populations of woylies such as those in the WA wheatbelt. Anecdotal evidence also indicates that the decline of woylies at Venus Bay Peninsula (VBP) may be related to drought and extreme weather events in association with cat predation.

A more detailed and rigorous assessment of climate changes and differences associated with woylie population declines is required. The differences observed between populations may be indicative that the declines observed in South Australia are to some extent due to different causal relationships from those in Western Australia, however, it is far too premature to regard this as anything beyond purely speculative.

7.2.4.2. Fox predation/scavenging

Fox predation and habitat loss/modification have historically been attributed as the key agents for the substantial range contractions and woylie declines across the Australian continent during the 19th and 20th Centuries. The subsequent recovery and re-establishment of woylie populations since the 1970s in the presence of fox control provides compelling evidence that foxes have indeed been a primary agent in historic decline of this and other species (e.g. Burbidge and McKenzie 1989; Kinnear 1994, Kinnear *et al.* 1998, 2002; Start *et al.* 1995).

Fox predation/scavenging was associated with the mortality of 15% of the radio-collared woylies in the Upper Warren region (i.e. comparable to the incidence of raptors and a third of the incidence of cats). None of these fox cases were associated with the woylie mortalities observed at Balban (i.e. during which 81% declines occurred in 12 months). Nonetheless, it is not possible to know the extent to which fox predation/scavenging was associated with woylie declines elsewhere within the Upper Region, given that they occurred in the absence of suitable monitoring data (i.e. no survival and mortality investigation of woylies and extremely limited available data on predator activities).

It is likely that fox densities have been highly variable over time in the Upper Warren region and elsewhere. This is particularly so given the likelihood for extended periods when fox control may have had reduced effectiveness (i.e. long interval times, timing associated with heavy rainfall events, bait interference, timing suboptimal according to fox biology, etc). Despite this, there is no compelling indirect evidence of these possible fox fluctuations resulting in substantial periodic declines in other prey species, either simultaneously or sequentially to the woylie declines.

These factors considered, it remains possible but speculated to be less likely than other agents, that fox predation has been a primary cause of the woylie declines in the Upper Warren region. By comparison, fox predation/scavenging was associated with about half of woylie mortality events observed in a comparable and concurrent study being conducted in Dryandra and Tutanning (Nicky Marlow, pers. comm.). It is therefore possible that foxes may have had a greater role in woylie declines elsewhere. Substantially more investigation is required to understand the strength and nature of the association between foxes and recent woylie declines. Nevertheless, highly effective fox control is highly likely to be a critical success factor for the rapid and robust recovery of the woylie, which is necessary for the long-term viability and conservation of the species.

7.2.5. Likely primary agents of decline

7.2.5.1. Mechanics of decline

Increased mortality is a likely primary mechanism causing the declines. Evidence for this includes;

- Reduced survivorship of the Balban radio-collared cohort associated with concurrent declines.
- The observed rates of decline (25% - 95% per annum) have been greater than can be attributed by a complete failure of recruitment by reproduction (i.e. 16% - 20% per annum based on a life expectancy of 5-6 years).
- The sudden loss of previously frequently-trapped woylie individuals from the grids and transects associated with woylie populations currently undergoing declines.

Emigration is highly unlikely based on there being no evidence of substantial movement and relocation of radio-collared woylies at Balban in association with the woylie declines. Furthermore, although further work is required, preliminary exploration of the extensive trapping data for the region has not detected any evidence of significant movements by substantial numbers of woylies.

Quantifying recruitment by reproduction into the adult population is inherently problematic. Based on the proportion of adult females with pouch-young recorded present, breeding does not appear to differ in association with spatial and temporal changes in woylie populations. However the power and sensitivity of these statistical inferences are yet to be quantified. The prevalence of subadults in the populations were very low and therefore the limited sample sizes collected at declining and declined populations have so far prohibited reliable statistical inference. The incidence of new (trapped for the first time) animals as a surrogate for adult recruitment has not yet been adequately examined but is also likely to be problematic due to low capture rates associated with declining and declined populations. Therefore it is premature to speculate or determine to what extent changes of recruitment into the adult population may be associated with the woylie declines.

7.2.5.2. Cat predation

Preliminary evidence that cat predation may be associated with woylie declines in the Upper Warren region include;

- Higher levels of cat activity associated with concurrent woylie declines at Balban.
- Cat predation was associated with 65% of the mortalities of radio-collared woylies for which some factor(s) associated with mortality could be attributed (i.e. 1/21 mortality cases was inconclusive).

However, not all evidence is consistent with predation/scavenging being a primary, ultimate and/or sole agent of decline, including;

- No or limited evidence of sympatric prey species (e.g. quenda, koomal, large reptiles, etc) declining either simultaneously or sequentially (e.g. prey switching). Note: statistical associations of woylie declines with quenda and koomal (Section 3.3. Meta-analysis) require further investigation.
- No evidence of a substantial and/or rapid increase in cat density/activity that could potentially drive such a rapid decline in species that was previously at high densities and with a high reproductive capacity. However, caution is required given the limited data available to examine this in the absence of extensive routine monitoring of predators. More thorough examination of the limited available data from long-term research in the Upper Warren (Kingston Study and Perup/Bushrangers) and other possible sources elsewhere is needed.
- If/where there is an increased activity or density of predators associated with a woylie decline, it is still necessary to establish whether this association is related or coincidental, and particularly whether it is a cause or effect of the woylie declines (i.e. experimental testing or equivalent is required to confidently discriminate).
- It is difficult to attribute the extent of declines (i.e. 0-5% of former woylie densities) solely to a predator given the reduced efficiency of continuing to hunt a prey at extremely low densities, particularly when the spatial pattern of declines is indicative of nearby forest concurrently supporting higher densities of woylies. It would reasonably be expected that at low woylie densities, a predator would seek a more efficient hunting strategy; either switch to an alternative prey source and/or move to an alternative area with higher woylie densities. On this basis declines as great as 100% can not be readily explained by predation alone.

Based on the balance of available evidence, it remains entirely possible that multiple factors may be involved in the woylie declines. Conversely, there is no evidence or reason to expect that the woylie declines are the result of a solitary factor. Furthermore, other factors may be predisposing an increased vulnerability to predation and/or carnivores are opportunistically exploiting a moribund or carcass resource being made more readily available. It is possible that different and/or multiple predators are exploiting the more susceptible woylies in different circumstances. This is one explanation, for example, why foxes are predominantly (but not exclusively) associated with woylie mortalities in Dryandra and Tutanning and why cats are predominantly (but not exclusively) associated with woylie mortalities in the Upper Warren region and VBP

7.2.5.3. Disease

Preliminary evidence that disease may be primarily associated with woylie declines in the Upper Warren region include;

- The rates and temporal characteristics of the declines are consistent with an epidemic disease agent.
- The spatial pattern of the decline within the Upper Warren (i.e. epicentre approximately at the Perup Ecology Centre and radiating out to the periphery and the last remaining moderate-density populations) is consistent with an agent with limited mobility (e.g. transmitted by direct contact or a vector with limited mobility such as lice, fleas, ticks and those transmitted in faecal matter).
- The spatial pattern of the declines within southwestern Australia and South Australia, superficially may also be consistent with the spread of an agent of decline. Substantially more spatial and temporal analyses at multiple scales are required to substantiate this.
- It is possible that there is a density dependency relationship to the declines, given the largest and densest woylie populations appear to have been most affected. Declines have not been detected in many small, isolated, peripheral and very low-density woylie populations – although this may be an artefact of very poor sensitivity to detect population changes with small sample sizes. A density dependency relationship is consistent with

the epidemiology of some disease agents as well as other factors such as resources and predators.

- An exceptionally high prevalence of coat and skin problems were associated with the concurrent declines at Balban, compared with other PCS sites. The extent to which observer differences might account for some of this difference is yet to be determined. While these symptoms in themselves are unlikely to be directly responsible for animal mortalities they have the potential to be general indicators of stress and/or related to other factors that might be directly involved in the declines.
- An acute response by individuals to the agent(s) of decline, resulting in mortality, seems a particularly likely function of the decline. This is speculated using the balance of evidence including the general condition of the animals monitored being generally good; the sudden loss of previously frequently-trapped woylie individuals associated with woylie populations currently undergoing declines; and recovered bodies show no evidence of emaciation or poor condition that can be attributed to a chronic problem.
- *Toxoplasma* has been detected in the Upper Warren but not in the unaffected population at Karakamia. This (and the absence of introduced predators) is one of the key distinguishing discriminating differences to arise from the population comparison study that may provide associative evidence of a potential agent of decline.
- The change in the prevalence of *Toxoplasma* in the Upper Warren region over time (i.e. 6% to 0%) is superficially consistent with the epidemiology of an agent of decline.
- The *Trypanosoma sp. nov.* prevalence and infection rates are likely to be another key distinguishing discriminating difference between Karakamia (stable) and Upper Warren (declined) woylie populations.
- Pathological (synergistic) interactions between *Toxoplasma* and *Trypanosoma* have been demonstrated in other species and circumstances, and therefore have the potential to be involved in some way with the woylie declines.

Limitations in attributing disease as a key agent of woylie declines include;

- Symptoms (gross, haematological, etc) of disease have not been recognised except for the potential association of poor coat and skin condition at the declining Balban site.
- There have been mortalities of apparently grossly healthy woylies at both the Upper Warren, and Dryandra / Tutanning that evidently do not involve predation or physical trauma. In all of these cases the cause(s) of death has been inconclusive.
- Necropsies and pathology have not detected evidence of disease(s) recognized as potentially capable of being associated with the woylie declines. The ability to do so has been substantially compromised by the limited woylie remains submitted for examination. The loss and/or consumption by predators/scavengers of all or most of the vital organs necessary for diagnosis means that in almost all cases it has been impossible to examine for diseases.
- There are hundreds of known diseases that potentially may be involved in the woylie declines, many of which require complex, problematic and/or specific (and often expensive) tests to identify their presence (e.g. many viruses).
- No direct and definitive tests of specific disease agent(s) to examine their capacity to reduce the fitness, condition, reproduction or survival of woylies have yet been conducted.
- Understanding the potential role(s) of disease in the woylie have been substantially constrained by the depauperate knowledge-base of what wildlife diseases are present in the woylie and other Australian wildlife, as well as their prevalence, ecology, epidemiology, pathogenicity and other host impacts (e.g. behaviour, reproduction, condition, etc), etc.
- Consideration of the unknown is necessary given that it is possible that disease agents currently unknown in this context may be involved.

7.3. Speculative hypothesis of the cause(s) for the recent woylie declines based on preliminary information

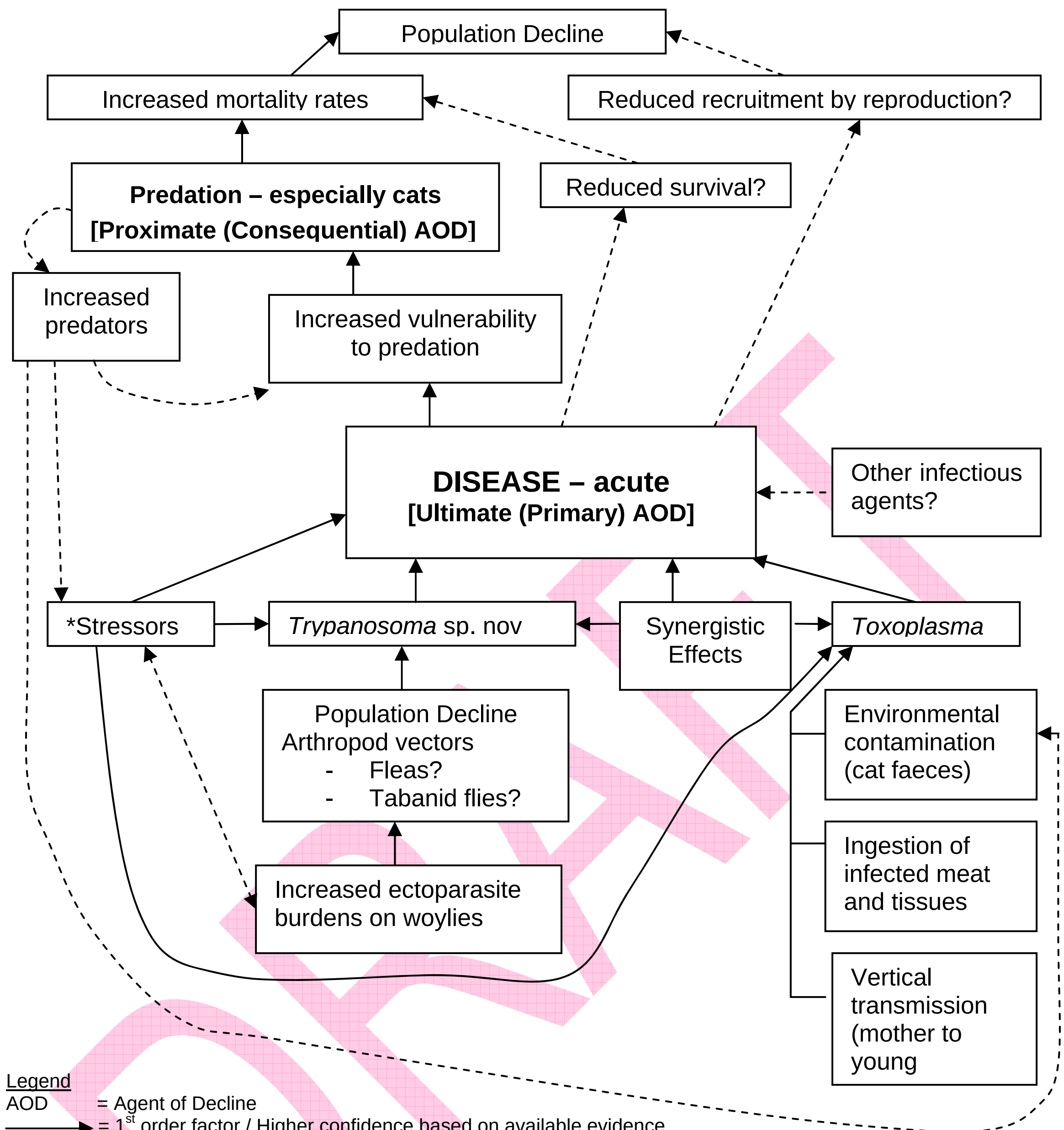
In summary, based on the preliminary evidence available for consideration, there is no single or simple compelling case evident as yet that can be strongly inferred as the fundamental cause(s) for the recent woylie declines. This is however, a ‘work in progress’ and there remains a substantial amount of work required to process, analyse, interpret and synthesise the data and information that has been collated and generated to date. Continuing this work should remain the highest priority given the investment to date and the anticipated returns for doing so. Despite the premature nature of the analyses it is possible to speculate and present the leading hypothesis of what may be causing the woylie declines based on the balance of emerging information. Again, it must be emphasised that this speculation be regarded appropriately given that it has not been rigorously tested.

The most credible hypothesis for the recent woylie declines can be summarised as;

- Driven by adult mortality at least in part, probably by an agent(s) ultimately operating acutely on individuals. Changes in recruitment by reproduction may or may not be involved. Loss by emigration is not likely.
- Result from multiple factors operating either simultaneously or sequentially.
- Disease(s) are the most likely primary or ultimate agent of decline. These disease(s) may be either novel or previously present but now function within a novel context of other interacting factors that have precipitated the recent woylie declines (Figure 7.1). The primary or ultimate agent(s) of decline are considered the critically important factor(s), without which, the death of an individual (in this case) or the decline of a population, would not otherwise necessarily occur.
- The available evidence is suggesting that *Toxoplasma* and *Trypanosoma* sp. nov. may be associated with the woylie declines (Figure 7.1) and are therefore the most likely agents of disease.
- Synergistic effects of *Toxoplasma* and *Trypanosoma* sp. nov. have been recognised in other host species (Guerrero *et al.,* 1997; Cox, 2001). It is therefore possible that synergism may also be occurring with woylies
- Other stress factors and/or infectious agents may also be contributing to the declines as either triggers to the development of disease or as concomitant agents.
- Possible stressors include predation, competition, climatic factors, extreme weather, concurrent infections, disease reservoirs in sympatric species, ectoparasites, nutrition, and high population densities.
- Other infectious agents of note that may in themselves be significant agents of decline include Chlamydiales, Macropod Herpesvirus, Orbivirus, Encephalomyocarditis virus and *Neospora caninum*.
- Predators/scavengers are likely to be consequently and opportunistically exploiting woylies made more vulnerable to predation/scavenging as a result of disease(s) (i.e. changed behaviour and/or reduced fitness, condition, and/or survival) (Figure 7.1). While cats are the dominant predator/scavenger in the Upper Warren, and Venus Bay Peninsula (VBP), foxes may be the dominant predator scavenger elsewhere (e.g. Dryandra and Tutanning). Predation/scavenging is considered a ‘proximate’ agent of decline, because it is more immediately associated with the fatality.

- The woylie declines at VBP, South Australia although characteristically similar in some respects to those observed in Western Australia, probably result from agents of decline that differ to some extent.
- Based superficially on the characteristics of the decline, the primary and ultimate agents of decline operating in the Upper Warren are probably the same as those operating in Batalling. Whether they are the same as those in Dryandra is less certain given the earlier commencement of the declines and the apparently more linear and slower rates of decline at Dryandra.

The leading hypothesis presented here and summarised in Figure 7.1 can serve to focus future research priorities and efforts to test its validity. It should also serve as a point of collegial discussion and development tool to refine our understanding of the potential factors. Nevertheless, given that it remains speculative and untested it is also important that research resources and efforts should not be directed exclusively to the primary hypothesis. Other valid lines of enquiry that test alternative hypotheses are also necessary at least until more compelling evidence for the cause(s) are established. Having said this, the available evidence and information accumulated in this initial phase of enquiry can be used to develop a narrower focus than has been used in the initial phase of this endeavour. It also remains important to the expedient success of the research to remain flexible and responsive, within a critical and precautionary framework, to new and emerging evidence.



Legend
AOD = Agent of Decline
——▶ = 1st order factor / Higher confidence based on available evidence
- - - -▶ = 2nd order factor / Likely relationships but less evidence available

Not primary factors of decline:
Habitat loss/modification
Fire
Direct human interference (live trapping)
Food resources

Population change – mechanical algorithm for Upper Warren Woylie Population:

0 -ve? 0 +++
Recruitment (immigration + reproduction) – Loss (Emigration + Mortality) ⇒ Population decline

*Possible stressors include:
Predation
Competition
Climate factors / Extreme weather
Concurrent infections
Ectoparasites
Nutrition
Disease reservoirs in sympatric species
High density woylie populations

Figure 7.1. The leading (untested) hypothesis of the causes of woylie declines in the Upper Warren region based on preliminary and untested inferences.

7.4. Acknowledgements

Not enough thanks can be given to the collaborative, supportive and generous spirit of the very large number of people and organisations involved in the WCRP. This is the greatest success and asset of the project and provides the greatest ever potential to successfully diagnose the woylie declines and thus provide the best opportunity for a robust and sustained recovery and long-term conservation of the species. The achievements that have been made are a tribute to all of those involved, and testament to all that is good about the scientific and conservation management community and its ability to make great things happen.

Thanks to all those that have contributed to the development of data and evidence within the diversity of components associated with the project. Thanks also to the many and diverse conversations that have helped to develop the approach and our growing understanding of woylie ecology within the context of the current declines and the available evidence. Thanks to those that have provided critical comment on this section and its concepts, including Ian Abbott, Andrew Smith, Paul Eden, Andrew Thompson, Neil Burrows, and Keith Morris.

7.5. References

Burbidge, A. A., and N. L. McKenzie. 1989. Patterns in the modern decline of Western Australia's vertebrate fauna: causes and conservation implications. Biological Conservation **50**:143-198.

Caughley, G. 1994. Directions in Conservation Biology. Journal of Animal Ecology **63**:215-244.

Cox, F.E.G. 2001. Concomitant infections, parasites and immune response. Parasitology **122**: S23-S38.

Guerrero, O. M., Chinchilla, M. and Abrahams, E. 1997. Increasing of *Toxoplasma gondii* infections by *Trypanosoma lewisi* in white rats. Revista De Biologia Tropical **45 (2)**: 877-882.

Kinnear, J. 1994. Feral predators: the impact of the fox and the feral cat on native wildlife. Impact and control of feral animals in south-western Australia.

Kinnear, J. E., M. L. Onus, and N. R. Sumner. 1998. Fox control and rock-wallaby population dynamics - II. An update. Wildlife Research **25**:81-88.

Kinnear, J. E., N. R. Sumner, and M. L. Onus. 2002. The red fox in Australia - an exotic predator turned biocontrol agent. Biological Conservation **108**:335-359.

Start, A. N., A. A. Burbidge, and D. Armstrong. 1995. Woylie recovery plan. Wildlife Management Program No.16. Western Australian Department of Conservation and Land Management and South Australian Department of Environment and Natural Resources, Perth.

CHAPTER 8 INTERIM / PRELIMINARY RECOMMENDATIONS

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Abstract

While the Woylie Conservation Research Project (WCRP) is ongoing, some interim and preliminary recommendations for research, fauna monitoring, conservation management and project management can be outlined. The principal research framework includes;

1. Phase 1 completion and synthesis
2. Key putative agents of decline
   a) Disease - Toxoplasma, Trypanosoma, Supporting (diagnostic) evidence (field, clinical, pathology, epidemiology), Other infectious agents (including priority risks), and Dependent follow-up (e.g. synergistic effects)
   b) Predator control experiment (Active Adaptive Management)
   c) Resources
   d) Stressors
3. Species recovery
   a) Conservation genetics
   b) Small population paradigm – limiting factors and risks
   c) Population viability analysis
4. Research management (statistical analyses support, reviews, symposium and workshop, data and information management)

A six-month plan for the completion of the current commitments to the WCRP is summarised.

Fauna monitoring recommendations include;

- Suggested programs for Upper Warren and elsewhere
- Improved monitoring protocols (surveillance versus ecological monitoring; target species, predators, other covariates; and trigger points, reporting and response protocols)

Interim and preliminary conservation management recommendations include;

- Conservation status reviews
- Recovery planning
- Improved efficiency to the current fox-control program
- Data and information management improvements

Project management considerations include adopting the appropriate framework for an ongoing program (as distinct from the rapid response approach of phase 1), consider the sustainability of the project (personnel to meet workload, resources, etc), support and funding, communication, data and information management, media and public engagement and the value of external and internal reviews.

Introduction

As originally planned, phase 1 of the Woylie Conservation Research Project (WCRP) is expected to conclude on 30 June 2008. The intent is that the work achieved in this initial phase will inform what should happen next. The second phase of the woylie conservation response should be distinct from the first in three important ways;

1. The project management should shift from the rapid (emergency) response model to an ongoing program model
2. Research focus shift from investigative to scientific testing
3. Response emphasis should begin to shift from research toward management

While the WCRP is ongoing, a framework of interim and preliminary recommendations has been provided to stimulate further development and facilitate the maintenance of momentum into phase 2. These considerations are broadly classified as

1. Research
2. Fauna monitoring
3. Conservation management
4. Project management

Figure 8.1 provides a higher-order outline for the Woylie Conservation Phase 2 framework.

8.1. Research recommendations

Based on the available evidence and the leading hypothesis of the cause(s) of the woylie declines (Section 7.3), a framework for future research is grouped into four major themes;

1. Phase 1 completion and synthesis
2. Key putative agents of decline
   a) Disease
      -Toxoplasma
      -Trypanosoma
      -Supporting (diagnostic) evidence (field, clinical, pathology, epidemiology)
      -Other infectious agents (including priority risks)
      -Dependent follow-up (e.g. synergistic effects)
   b) Predator control experiment (Active Adaptive Management)
   c) Resources
   d) Stressors
3. Species recovery
   a) Conservation genetics
   b) Small population paradigm – limiting factors and risks
   c) Population viability analysis
4. Research management

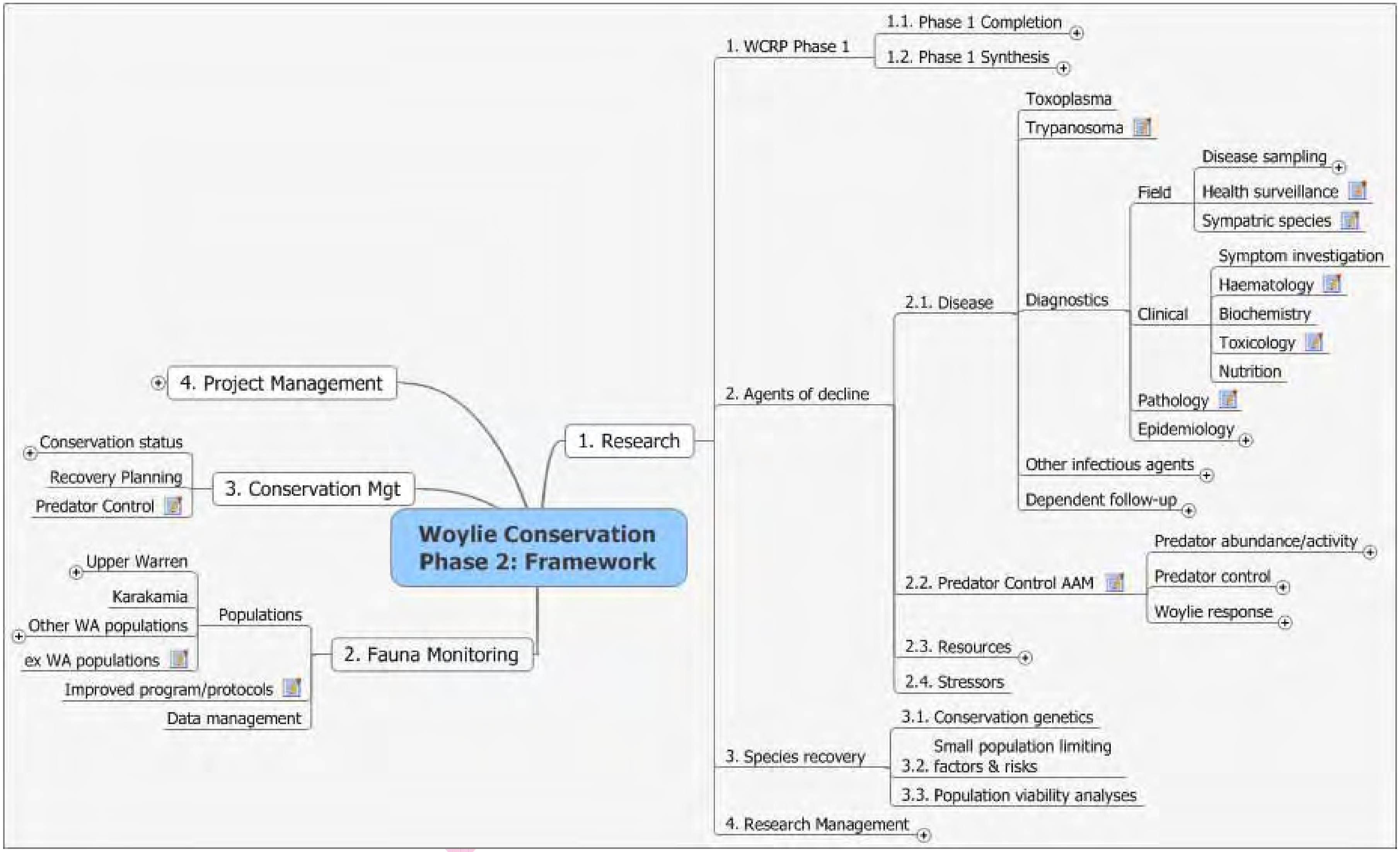

Figure 8.1. Draft framework for the development of Phase 2 of the woylie conservation response plan.

Shifting focus for new research

Research to date has focused on finding associative evidence related to recent woylie declines. While this has been the most efficient preliminary strategy available to determine what primary factors may be involved they provide relatively weak inference. This is because associations may be either *related* or *co-incidental* to the declines. Furthermore, it is necessary to discriminate between related associations that are a *cause* of the declines from those that are an *effect* (i.e. a consequence or response to woylie declines). In so doing, the next phase of research should serve to progress the woylie decline diagnosis framework (*sensu lato* 'declining population paradigm'; Caughley, 1994; Caughley and Gunn, 1996; Peery *et al.,* 2004) by concluding steps 1-5 and shifting focus onto step 6 and 7;

1. Confirm that the population has declined.
2. Determine the spatial, temporal and demographic characteristics of the observed decline.
3. Understand the species' ecology.
4. Identify all potential causes.
5. Use circumstantial evidence to help shortlist the potential causes.
6. Seek direct evidence – test putative causes.
7. Given the evidence, determine the most appropriate conservation and management responses within an active adaptive management framework.

Scientific experimental testing is highly recommended as the next stage in determining the factors associated with the recent woylie declines. Such an approach provides the scientific rigor necessary to test the nature and role of the putative factors that have been identified by association with woylie declines. Scientifically testing these associations is a particularly critical next stage given the potential risks of undertaking management and conservation responses based on untested hypotheses derived from associative evidence. The extinction of the California Condor in the wild in the 1980s, is an example where conservation and management efforts based on untested theories unwittingly facilitated their decline (Caughley, 1994).

An active adaptive management framework (*sensu* Walters and Holling, 1990 and Lee, 1999) is recommended as the scientific framework to test putative agents of decline wherever possible and appropriate. This 'learn by doing' approach helps address the need to respond to the declines in a timely manner and to optimise the conservation prospects for the species (e.g. minimise loss of genetic diversity, limit vulnerability to declining populations to local extinction, facilitate rapid recovery, etc). Such an approach can also serve to minimise the risks of pursuing actions that unintentionally have adverse affects.

Flexibility and responsiveness to new and emerging evidence remains a necessarily important provision given the nature and complexity of the situation.

8.1.1. Limited continuation of elements of WCRP phase 1

8.1.1.1. Phase 1 - Completion of existing work-in-progress

The collection of primary data in the field was predominantly concluded by August 2007 for all components of the project with the exception of a reduced-level of on-going Upper Warren Fauna Monitoring, resources PhD research, and the opportunistic collection of some samples for ongoing disease investigations (discussed more below). The emphasis since then has been to process and analyse the available samples and data.

The continued processing and analyses of data is expected to provide substantial and important information necessary for diagnosing the woylie declines. This continued work includes;

Meta-analysis

- Spatial and temporal characterization of the Upper Warren declines

- Population change associations with other species needs resolution for koomal, quenda and large reptiles and investigation of the more cryptic ngwayir, wambenger and possibly numbat.
- Consideration of expanding the meta-analysis (population trends, demographics, other associations, etc) to include analysis and comparison with other woylie populations (e.g. Batalling, Dryandra, Tutanning, and South Australian populations).
- A more comprehensive analysis of climatic associations with the woylie declines.

Survivorship and mortality (PCS)

- Complete comparative disease and health profiles of radio-collared woylies that died/survived.
- Complete evidence processing of forensic material and necropsies collected from woylie mortalities
- Complete evidence processing of material collected from the woylie 'search and rescue' exercise. Consider another community-based exercise.

Peer-reviewed publications

The population comparison study components and disease investigations should complete the analyses of existing data currently underway, and publish the works directly into the peer-reviewed literature. A publications plan and register of all material derived from the project and its collaborative association is being maintained and is available to all WCRP collaborators. It is expected that manuscript submissions will begin before mid-2008.

Incomplete or outstanding / Low priority work

Meta-analysis elements that remain incomplete but lower priority include;

- Demographic analysis of population turnover and individual longevity
- Fire associations with woylie declines

Population comparison study elements that remain incomplete but lower priority include;

- Demographic analysis of population turnover and individual longevity
- Wedge-tailed eagle predator abundance and nest surveys
- Dietary analysis of predator scats collected during the sandpad surveys
- Analysis of historical sandpad survey data from the Kingston Study and Perup Bushrangers program
- Resources pilot study analysis

8.1.1.2. Phase 1 - Synthesis of existing evidence

Once the analyses of the various components have been sufficiently advanced, it will be necessary to undertake a comprehensive synthesis of the available information. A critically necessary progression in the development of the evidence is relating and linking the multiple datasets available to identify associations with individuals/populations at different stages of decline.

Key datasets include;

- Survival and mortality radio-collared cohorts
- Trapping data from PCS sites
- Upper Warren monitoring transects
- WA and SA populations

Key data types include;

- Disease incidence/prevalence
- Clinical indicators - haematology, biochemistry, etc

- Pathology - necropsies
- Field health examinations
- Biometric and trapping
- Predator activity

These lines of enquiry are related to the epidemiological investigations and the development of disease/health profiles discussed further below.

The synthesis of available information will include the consideration of developing systems models to assist understanding and communicating the likely interactions and complexities associated with the woylie declines.

8.1.1.3. Close monitoring and study of the last moderate-density woylie populations

Keninup, and to a lesser extent Warrup, constitute the last remaining wild woylie populations still persisting at moderate densities in Western Australia. There is every expectation that Keninup will undergo substantial and rapid declines during 2008. This is based on its close geographical association to the latest decline at Balban (81% decline in 12 months to July 2007 and continuing) and the pattern and characteristics of the declines observed throughout the region. This provides the best and possibly last opportunity to directly identify the cause(s) of woylie declines so that appropriate conservation and management can effectively promote the recovery and long-term viability of the species. The importance of this opportunity is particularly well emphasized by the immeasurably greater difficulty to identify these factors in low-density woylie populations and after the decline has occurred. The factors that keep a population low also may not be the same factors that cause the initial declines.

Continued close monitoring and study of the woylie populations at the Population Comparison Study (PCS) sites is highly recommended. This is especially so for Keninup, Warrup and Karakamia – which remains a particularly powerful comparative population. Comparative data from a population that has declined would also be necessary to assist in the diagnosis of the woylie declines.

8.1.2. Key putative agents of decline

The research framework for the key putative agents of decline are grouped into four themes;

a) Disease

b) Predator control experiment (Active Adaptive Management)

c) Resources

d) Stressors

8.1.2.1. Disease

Toxoplasma

- Spatial and temporal differences in *Toxoplasma* evidence in relation to woylie population declines. Sources of evidence include serology from blood samples, PCR and histopathology of available tissues from dead radio-collared woylies and necropsies.
- Epidemiological investigation to relate evidence of *Toxoplasma* infection with other available disease, health, demographic, and survival data, compared with individuals/populations not infected.
- Genetic characterization
- If the associative evidence for *Toxoplasma* having a role in the woylie declines is compelling, several more lines of enquiry should be pursued including; evidence of toxoplasmosis, possible synergisms (e.g. *Trypanosoma*), virulence testing, relative importance of the various transmission pathways (cat faeces, infected meat, vertical transmission), etc

Trypanosoma

- Spatial and temporal differences: comparison of binary PCR data (positive/negative) within (across time) and between (across space) populations. If there appears to be a link, or role, for trypanosomes then a more in-depth modelling analysis will be required for significant publication
- Genetic characterisation
- Epidemiological investigation to relate evidence of *Trypanosoma* infection with other available disease, health, demographic, and survival data, compared with individuals/populations not infected.
- Parasitemia (in association with QPCR if possible)
- Experimental infection - will provide the most compelling evidence for the pathogenic status of trypanosome infection. Could be expanded to include the concomitant effects of *Toxoplasma*.

Supporting (diagnostic) evidence

Field sampling and data collection includes;

- Disease sampling at PCS sites and Upper Warren fauna monitoring transects should continue (blood, scats, ectoparasites, DNA) for the development of the sample reference bank (i.e. capacity to retrospectively examine compelling new evidence) and to examine spatial/temporal differences in disease prevalence associated with declines.
- Disease sampling at PCS expansion sites (Batalling, Dryandra, Tutanning, etc?) to provide key discriminating associative evidence for the possible role of *Toxoplasma*, *Trypanosoma* and other possible infectious agents associated with the woylie declines.
- Ongoing field health surveillance of woylies and other fauna caught in the Upper Warren, other swWA and SA populations (i.e. primary means of detecting clinical symptoms that may provide critical breakthroughs to the diagnosis of woylie declines).
- Disease sampling of sympatric species by the associated ARC linkage project may provide useful leads.

Clinical activities providing supporting evidence includes;

- Clinical investigation of symptomatic evidence in sick or moribund animals
- Haematology
- Biochemistry
- Toxicology (e.g. sodium monofluoroacetate, alkaloids, and heavy metals)
- Nutrition

Pathology investigations, including detailed necropsies to maximise the capacity to identify and collect evidence that may be associated with the causes of woylie mortality and population declines.

Epidemiological investigations and characterisation of the decline would be particularly powerful tool to assist in the diagnosis of the woylie declines. Such an investigation may help to identify how and what diseases may be involved in the declines based on the evidence accumulated to date. Investigations could include;

- Comprehensive aggregation of all available data collected to date on the disease/health profiles of the survival and mortality radio-collared cohorts (died versus survived), PCS sites, and broader comparisons between other woylie populations.
- Research and synthesise all available disease and health information in the published literature and other available records relating to woylies and related species.
- Comparison between observed and predicted behaviours of key putative agents and mechanisms of decline
- Review the identification and risk assessment of potential disease agents in relation to woylie declines in an ongoing or at least annual basis.

Other infectious agents

- High priority / high risk infectious agents that have not yet been investigated include Macropod Herpesvirus, Orbivirus, Encephalomyocarditis virus, Chlamydiales bacteria, and the protozoan: *Neospora caninum*.
- Ectoparasites
- Bacteria
- Endoparasites
- Viruses
- Haemaparasites

Dependent follow-up

Dependent on anticipated and/or more compelling evidence, there are a number of investigations that may be considered as logical developments. These include;

- Synergistic effects – particularly between *Toxoplasma* and *Trypanosoma*
- Testing the role of associated diseases (transfection study)
- Arthropod vectors of associated diseases

<u>Synergistic effects</u>

Investigations into possible synergistic effects would commence once associative evidence of *Toxoplasma* and *Typanosoma* both being involved in the woylie declines has been established and/or if other potential synergistic effects are well founded.

<u>Testing the role of disease – 'transfection' study</u>

There is mounting evidence that woylies from different populations differ in the prevalence of *Toxoplasma* and *Trypanosoma*. Little is known about the mechanism of transmission of *Trypanosoma* and its potential for pathogenic effects on its woylie host. The aims of this project are therefore (1) to determine the role of arthropod vectors in trypanosome transmission; (2) to determine the extent to which trypanosome infections may adversely affect woylie health and/or condition and/or activity in a manner that may contribute to woylie population declines. Possible interactions with *Toxoplasma* and other key disease agents can also be investigated as part of these trials.

The primary principle of the experimental investigation is to co-house, in a controlled environment, woylie individuals from an affected population (Upper Warren) with naive individuals from a population unaffected by the recent declines. The ectoparasite loads would be removed from woylies in some of the concurrent trials to determine their role as vectors. Close and frequent observation of the animals by suitably trained individuals and supervision by veterinarians would monitor the health and behaviour of the animals. The ethical considerations and welfare of the animals involved will remain an utmost priority and will comply with all the necessary codes and requirements in the most appropriate manner.

The aim would be to conduct this research as a PhD student project and as a collaboration lead by Murdoch and involving DEC, possibly Perth Zoo and others. A/Prof Alan Lymbery and Prof. Andrew Thompson have had lead roles in the development of this project proposal. The plan is to begin the project in 2008, running for a minimum of two years. Enclosures would be required to accommodate the woylies involved and a not-for-profit community organisation has been engaged as prospective key collaborator in this capacity. Funding for the research is currently being sought.

<u>Arthropod vectors to disease (student project)</u>

Given the possible association of trypanosome infection with Upper Warren woylie declines there is a need to understand what vector(s) may be involved in transmission of this parasite. One possibility is the ectoparasite assemblage that live on woylies (fleas and ticks), but trypanosomes are commonly transmitted by biting flies of a number of species. Cyclical development can occur in the vector, either in the anterior of the insect with subsequent accumulation in mouthparts and salivary glands and transmission by biting, or in the hindgut with passage by faeces resulting in contamination of mucous membranes or wounds.

The objective of this study would be the examination of collected or trapped vectors for presence of the trypanosomes.

a) Existing activity is already planned to examine ectoparasites removed from the animals (fleas, ticks, mites) for evidence of infection by trypanosomes and *Rickettsia* (i.e. Halina Burmej's PhD research).

b) The proposed additional activity is to:

- set insect traps in the Upper Warren and at Karakamia
- detect and sort species of biting flies that are trapped
- examine mouthparts/salivary glands and hindgut for presence of trypanosomes by direct microscopy and/or by PCR

This project has been identified by the Woylie Disease Reference Council (WDRC) as one of the priorities for new potential research and has been developed and championed by Dr Trevor Ellis and Dr Phil Nicholls. The project would be suitable for an Honours student project supported by a DEC field entomologist (e.g. Janet Farr) and parasitologists at Murdoch University. It will assist in determining the differences in incidence and prevalence of trypanosomes between woylie populations and could help to understand the epidemiology involved. The priority of this research would be elevated if additional evidence indicates that Trypanosomes or other vector-borne diseases potentially transmitted by biting flies are in some way related to recent woylie declines.

8.1.2.2. Experimental introduced predator control within an active adaptive management framework

The remnant woylie populations within the Upper Warren region are well-suited to experimentally testing the role of introduced predators in current woylie declines. Given the spatial distribution and status of woylie populations within the region, the approximately 50 km by 50 km area could be used as an unreplicated experimental trial in which part of the area is managed as a control (existing predator management) and the another portion as the treatment area (increased and sustained cat and fox control).

Summary points of the proposed experimental design

<u>Control area (predator management unchanged):</u>

Western and Southern portion of the Upper Warren region, including the Proposed National Park in the greater Kingston area and State Forest immediately south (Figure 8.2). This includes Warrup which supports one of the two last remaining moderate-density wild woylie populations. Both the Warrup and Winnejup (declined woylie population) areas have long-term medium-sized mammal data, and more recently, more detailed data associated with Upper Warren woylie monitoring and PCS sites (including woylie grids and predator sandpad networks). The area is also involved in ongoing research which would otherwise be disrupted if it were to be the treatment area (e.g. Kingston Study and FORESTCHECK).

<u>Treatment area (intensive, aggressive and sustained cat and fox control):</u>

Northeastern portion of the Upper Warren region, including all or most of the Perup Nature Reserve (Figure 8.2). This includes Keninup which supports the most substantial of the two last remaining moderate-density wild woylie populations as well as representative areas that have recently undergone >90% declines in woylies. The area also includes proposed reintroduction sites for bilby and boodie and constitutes important habitat for the indigenous populations of the threatened ngwayir and other threatened mammals that can be predated by cats and foxes.

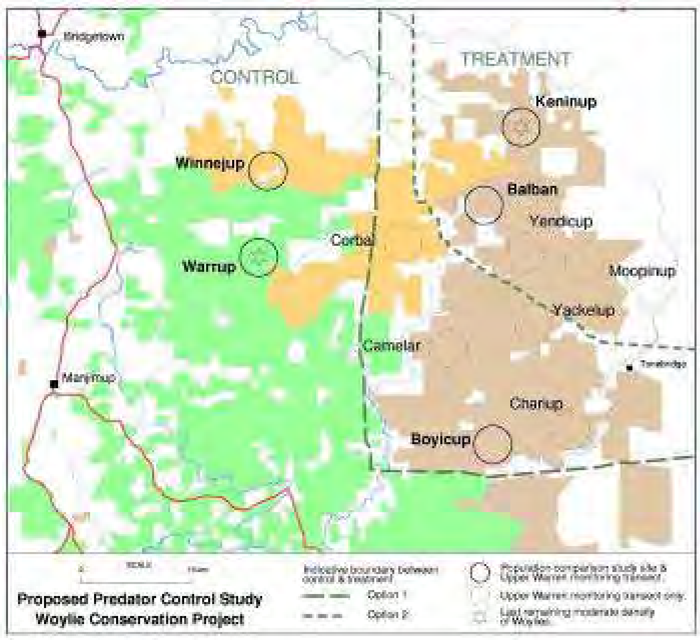

Figure 8.2. Proposed Predator Control study using an experimental and active adaptive management design that directly tests the role of predators (principally cats) in the current declines of woylies in the Upper Warren region.

<ins>Predator control:</ins>

Intensive and sustained cat control would use one of two approaches that both aim to maximise the reduction of feral cats in the treatment area and sustain them at these levels over time.

Option one: Cat control using methods that can directly quantify the number of cats removed (e.g. trapping, shooting, rapid-kill baiting).

Option two: Cat control using methods that do not directly quantify the number of cats removed (e.g. broadscale baiting).

Option one is the vastly preferred approach given that it more directly quantifies the reduction in cats, which can then be directly related to woylie responses. A substantial and significant additional benefit of this approach is the ability to calibrate and test the sensitivity of methods used to monitor predator activity and/or estimate predator densities within the southwestern Australian forests, which is notoriously difficult and problematic. The support and expertise of Dave Algar and others would necessarily be a collaborative component of this program. As well as being directly beneficial to woylie conservation efforts this study would also have broader benefits and relevance for predator control and monitoring across the State and elsewhere.

<ins>Predator monitoring:</ins>

Fox and cat activity and abundance would be regularly monitored before and during the study in both the control and treatment areas to quantify treatment effects and changes in predators over time. Predator control option one would enable the monitoring methods to be calibrated and their sensitivity quantified as discussed above. Monitoring methods would include;

- Sandpad surveys using the networks established as part of the WCRP. These will be used principally to measure activity and possibly abundance of cats and foxes, as well as native fauna.
- Hair traps for DNA mark-recapture abundance estimates (currently being developed and refined by Mesopredator research colleagues Paul de Tores and Nicky Marlow).

Woylie and other native fauna monitoring

Existing PCS (grids) and Upper Monitoring infrastructure (transects) will be used to quantify woylie and other native species responses to the increased predator control using a similar program as conducted in 2006/2007. This will include studying the responses to increased predator control by;

- Regular trapping on the PCS grids to study density and demographic responses.
- Radio-collared cohorts in the treatment and control areas will be used to quantify survival and mortality.
- Upper Warren Fauna Monitoring transects will provide landscape-scale responses.
- PCS grids and UW Fauna Monitoring transects will also quantify responses by other native species.

Other points for consideration

- This project will necessarily be a highly collaborative project providing mutually beneficial outcomes and accessing the expertise of mesopredator research colleagues, particularly in relation to predator control methods and hair traps for DNA mark-recapture abundance estimates.
- The project could provide a continuing leading example of a high level of co-operation between operational and research components within the Department. The operational components of the study (e.g. predator control and landscape-scale monitoring) would be well-suited to existing and new works managed by Regional Services and Nature Conservation Divisions. Science Division can provide scientific and specialist support.
- This project is well-suited to be incorporated into the suite of Departmental active adaptive management projects currently being established.
- The relatively high reproductive potential of woylies means that if predation is a key factor in the decline of woylies, population responses should be detectable within one to three years.
- The survival and mortality data generated from monitoring radio-collared cohorts should provide strong (partial) evidence within the first 12 months of intensive predator control
- Factors that cause the decline of a species may be different to those that then keep populations at low levels. Predation is likely to play an even more important role in the former. Therefore this project is expected to be a potentially key contributor to the optimisation of the recovery of the species, which will critically have substantial long-term conservation benefits (i.e. minimisation of genetic loss due to a large and protracted bottleneck).
- The greatest genetic diversity of woylies is expected to be at the remnant indigenous populations at Upper Warren, Dryandra and Tutanning, of which the latter is likely to be limited value given the relatively small population size and historical isolation. Conservation genetics research currently underway by Carlo Pacioni (Murdoch University) will quantify this in 2008.
- The proposed predator study is best suited for the Upper Warren region given the existing infrastructure in the field (e.g. transect, grids, sandpad networks) and office (e.g. Manjimup-based inter-divisional collaborations), the substantial foundation of existing data and information from >30 years research and monitoring activities, including the current WCRP, relative value of the population as one of the three remnant indigenous population (i.e. highest conservation value), and geographic attributes of the region (size, proximity to Departmental resources).

8.1.2.3. Resources

Resources investigations undertaken as part of Kerry Rhodda's PhD research should continue. As well as scientifically assessing whether or not food resources are associated with the woylie declines, this research will provide important ecological information directly relevant to the recovery and long-term conservation of the species.

Vegetation health, structure and floristic data from the resources pilot study and PCS sites remains to be analysed. Similarly the analysis of woylie digging densities and associations from the resources pilot study remain to be completed.

8.1.2.4. Stressors

Stressors that may trigger or contribute to the effects of key agents of decline include predation, ectoparasites, competition, nutrition, climate factors / extreme weather, disease reservoirs in sympatric species, concurrent infections, and high-density woylie populations. Researching and monitoring these and other possible stressors should be considered. The importance of understanding the potential stressors will increase as the compelling evidence for the key agents of decline accumulates.

8.1.3 Species recovery

As well as being relevant to the diagnosis of the causes of the woylie declines, some research is particularly important for facilitating rapid and appropriate species recovery. This includes;

8.1.3.1. Conservation Genetics

A good understanding of woylie population genetics is important for a number of reasons including; characterisation of the remaining genetic diversity, understanding the consequences of previous bottlenecks thus enabling predictions of the consequences of the current declines, and informing how best to facilitate the recovery of the species in a manner that will maximise the genetic diversity and hence maximise the long-term conservation potential of the species. This work is being conducted as part of a collaborative PhD research program by Carlo Pacioni.

8.1.3.2. Small population paradigm

Understanding the limiting factors and risks to small populations are particularly important given that they can be profoundly different to declining populations. This is therefore an important complement to the diagnosis of the causes of the woylie declines.

8.1.3.3. Population viability analyses

Population viability analyses will help characterise the nature of the declines, quantify the impact of the declines, assess the risk to populations and help instruct recovery plans and management to maximise woylie conservation prospects.

8.1.4 Research management

8.1.4.1. Statistical analyses

Conducting statistically rigorous and appropriate analyses remains a key limiting factor in the progress of many of the WCRP component investigations. This is due to the extensive competing demands of Adrian Wayne as the project manager and chief investigating officer and the absence of any other individuals with the appropriate skills to perform many of the necessary statistical analyses. This problem has now become increasingly important and critical to the project as the emphasis has now shifted to analysing and publishing the results to date. Involvement of a suitably-experienced biometrician would be singularly the most effective means of increasing the capability of the project to produce results in a timely manner. This will immeasurably benefit the diagnosis of the declines and improve the capacity to respond in a manner that maximises the conservation and recovery of the species. A workable solution to this situation is now one of the highest priorities for the progress of this project.

8.1.4.2. Project and research review

Potentially substantial gains could be had by inviting a critical review of the WCRP and its preliminary results (presented in this report). Such an exercise could help to identify what has been done well and what could be improved or reemphasised in terms of priorities. This would be particularly valuable given the urgency for a rapid response to the situation and the vast amount of work that has been conducted by considerably limited resources (particularly people and expertise). It would also be timely to be conducted now so that it can contribute to the considerations of what happens next (i.e. priorities, strategies, etc). The project and research review would have several components;

1. General research approach and progress – conducted by the Mesopredator External Review Panel (Andrew Burbidge, Peter Jarman and David Choquenot).
2. Specific disease components – conducted by invited experts from the Australian Wildlife Health Network (AWHN) and the Australia Wildlife Disease Association (WDA).
3. Project Management – communication and comparison with analogous programs, e.g. Tasmanian Devil Facial Tumour program.
4. Self review – workshop(s) involving those directly involved in the WCRP.

8.1.4.3. Woylie symposium and strategic review

This is planned for the 14th and 15th February 2008. The Woylie Conservation Steering Group will convene the symposium and workshop. The purpose of the woylie symposium is to aggregate and communicate of all relevant woylie information currently available. The intended audience includes the large number of collaborators involved in parts of the WCRP and mesopredator research programs, fauna conservation managers, relevant experts not directly involved with the current programs but may have some capacity to contribute (including specific invitees), and associated and interested community groups and individuals. The symposium will also inform the subsequent workshop, which will be attended by invitees (principally researchers and managers directly relevant to woylie conservation and decline diagnosis). The purpose of the workshop will be to use the current information available to agree on the priorities and a strategic woylie conservation response plan (phase two). This will be further developed and presented as a proposal for consideration by key DEC Directors and/or Corporate Executive so that it can be assessed on its merits and within the context of other competing priorities.

8.1.4.4. Data and information management

Research and operational protocols

The WCRP Field Operations Handbook (Volume 3) outlines the methodology and protocols for the various components of the project. Components of this handbook are of value to other research and operational programs. Whole or parts of the handbook have been provided to colleagues and other workers and are being used in their current form or as a basis of protocols and procedures in other programs. While functional in their current form, some of the details necessarily change in order to be responsive to the changing circumstances and emerging information. If/when further decline diagnoses and/or monitoring are conducted using this handbook, a minor revision will be required beforehand. Consideration should also be given to managing the handbook in a manner that facilitates occasional or ongoing revision. For example, the latest version should be available electronically and readily accessible by a geographically dispersed and institutionally diverse target audience (e.g. web-based). Reformatting into a more user-friendly format would also be highly recommended. For example, 'Mindjet MindManager' software organises the information into a self-structured organisational chart that can be intuitively and easily navigated to rapidly access the target information. Accessing and reading the protocols can be done using free Mindjet software.

Improved data collection efficiency

The use of PDAs or similar hardware and software that can substantially increase the efficiency and quality of data collection and management should be given careful consideration if/when further work is conducted as part of this or similar work. The potential efficiency benefits alone from this are potentially substantial, particularly within the context of limited personnel resources. Given that this is a common problem across many programs within the Department, there would be substantial gains from a co-ordinated, cross-Department consideration and development of the use of these tools.

Database aggregation / centralisation

Substantial benefits have been gained by the creation of a single, centralised, aggregated database ('Manjimup Fauna File' (MFF)) containing fauna data from previously isolated programs operating within the same geographic area (Upper Warren). The value of the database is substantially greater that the sum of the isolated parts from which it was created, not least because the same fauna individuals now have a more comprehensive and integrated record of their history and association across separate programs. The analytical benefits of this in assisting in the diagnosis of the woylie decline, or any other fauna-related work is potentially invaluable. Such an approach to data management is also clearly more efficient and highly desirable for many reasons, despite the considerable effort required to aggregate the databases.

Ongoing maintenance of the MFF is necessary now that the substantial investment has been made to establish it and given the demonstrable benefits that have and will come from its ongoing use. This should include keeping the database up-to-date and continue efforts to validate the data to ensure a higher quality of data with a minimal error rate. Co-ordination and co-operation between the multiple contributors/users (i.e. Warren Region, Donnelly District, *Western Shield*, and various ongoing and historic research programs conducted by Science Division) is also necessary.

Future development of the database would also be beneficial. This should include the obligation by future work to use the MFF as the primary data management platform - using a centralised database at the outset is vastly more efficient than attempting to aggregate a separate database at some later date. Other developments could include, aggregation of the remaining datasets associated with the region (e.g. other Science Division databases, and work by external institutions such as UWA, Landcare groups and community groups, etc), and the expansion of the geographic area that is managed by the database (e.g. whole of Warren region).

The work on the Upper Warren data aggregation provides a model for what could happen more broadly across the Department. As such it has already assisted in efforts currently underway to improve the *Western Shield* database, 'Fauna File'. The Upper Warren example should continue to support and encourage efforts towards a more Department-wide integrated fauna data management system.

8.1.5. Summary of the short-term WCRP plan and future work preparation

The emphasis of the WCRP for the remainder of the 2007/2008 financial year will be the continuation of analyses and work toward the publication of the results to date, in keeping with the original plans and agreements for this project. The existing funding and formal commitments to the WCRP will conclude on 30th June 2008. The outcomes of the work-to-date will, however, inform what further responses may be committed to, also in keeping with the original strategy. However, in order to facilitate a timely and effective transition from the end of phase 1 and the start of phase two, planning is required well in advance of this milestone (hence the need to publish this progress report at this time). An efficient transition is particularly important given the significance and urgency of the issue and the substantial time-dependent opportunity costs associated with identifying and responding to the rapid species decline. In this context a plan for specifically addressing this transition is proposed;

Jan08	WCRP Progress Report – circulation to collaborators, DEC Directors, BCI, Director General, etc
Feb08	Project progress review General – Mesopredator Review Panel Disease – Wildlife Disease Association (WDA Australasia) / Australian Wildlife Health Network (AWHN) Project management Self review
Feb08	Woylie symposium and strategic review workshop - Publications should be forthcoming from herein
Mar08	Woylie conservation response plan (phase 2) continued development - including priorities, resource requirements, funding strategy. Incorporates reviewer and workshop contributions.
Mar/Apr08	Upper Warren Fauna Monitoring Re-instate predator sandpad monitoring (highly recommended)
May/Jun08	Secure support, funding and commitments to phase 2
Jul08	Commence phase 2 This may include predator control management/experiment, study of the last moderate-density woylie populations, and/or ongoing and emerging disease investigations.

8.2. Fauna monitoring

Fauna monitoring is directly involved in existing and recommended research and management activities. As such this section is relevant to research and management recommendation sections (above and below).

8.2.1. Woylie Populations

Upper Warren Fauna Monitoring

Continue the fauna monitoring program albeit on a less intensive basis. At the very least this should remain biannual for the last remaining moderate-density woylie populations (Keninup and Warrup). All other sites could resume to their pre-WCRP monitoring frequency but retain the standardized operational protocols (Volume 3) to ensure consistency and comparability (i.e. annual for most – some in spring, some in autumn, biannual for Yackelup and Yendicup) (Chapter 2 UW Fauna Monitoring). Careful consideration should be given to annually surveying those key Upper Warren transects that would not otherwise be surveyed on this basis (i.e. Chariup, Winnejup and Corbal) until either the cause of the declines is identified, the trajectory of the conservation risk to the species is improved and/or a robust recovery can be demonstrated. Changing the frequency of trapping needs to carefully consider the consequences and potential confounding resulting from changed capture probabilities based on trap-learning by woylies and other species.

Predator monitoring at the Upper Warren PCS sites should be re-activated in early 2008 as a matter of priority, particularly given the likelihood of cat predation being involved in the woylie declines. This is particularly critical covariate data to have concurrently available with the imminently-expected woylie declines at Keninup and possibly Warrup. This would also provide pre-treatment predator activity data necessary for the predator control experiment (AAM) and remains consistent with recommendations for more comprehensive ecological monitoring.

Karakamia Wildlife Sanctuary

The Karakamia (AWC) woylie population remains important for reasons including; assisting in the diagnosis of the woylie declines, remaining as the only high density/high productive woylie population unaffected by the recent declines in Western Australia, the relative security of the population, and as a source for translocations. It is therefore important that monitoring and health surveillance remains ongoing, and that it continues to serve through close collaboration with AWC as the most powerful comparative population available to provide associative evidence for the cause(s) of the woylie declines.

Other populations

The monitoring and health surveillance of other woylie populations in Western Australia and elsewhere remains necessary to develop an understanding of the magnitude and characteristics of the population changes across the species range.

8.2.2. Improved fauna monitoring

Type of monitoring

The vast majority of fauna monitoring conducted by the Department, including *Western Shield* is surveillance monitoring. ‘Surveillance’ monitoring provides information on the changes of the targeted measures over time (in this case generally native fauna abundance). It does not provide any capacity to associate or explain observed changes with factors that might be causal agents of these changes.

Ecological or informed monitoring includes collecting comparable covariate data that can be used to help explain changes in the targeted measures, such as threatened species abundances. This additional information can help identify the mechanisms and characteristics of the change (e.g. changes in the rates of loss and recruitment, and population demographics, respectively) and provide associative data that can identify or eliminate the possible cause-effect relationships driving the observed changes.

In the case of the recent woylie declines, the surveillance monitoring was able to detect the changes in woylie abundances but provide limited or no data on the mechanics or associations that might help explain these declines. As a consequence there has been a significant and substantial lost opportunity in the ability to identify the cause(s) of the declines within a timely manner. The ability to rapidly mitigate these declines has been compromised and delayed.

Pragmatically, more informed monitoring is recommended in circumstances where it is considered there are sufficient conservation values and potential risks. For example, this may be merited for fauna populations of critically endangered, endangered or high conservation value and/or where there is reasonable chance or expectation that populations may change over time due to anticipated threatening process or stressors. Similarly areas that support a number of species of high conservation value and/or those subject to significant or multiple pressures should be considered for informed monitoring.

It is therefore recommended that fauna monitoring conducted by the Department be reviewed with respect to the value of improving the quality and type of monitoring undertaken in order to maximise the conservation and management outcomes and to increase the capacity to understand the ecology of these systems better. This remains relevant to many of the activities conducted by the department including species recovery plans, threatened ecological communities, *Western Shield* (predator control, translocations, and species conservation), management activities such as fire and timber harvesting (e.g. FORESTCHECK) and the effects of other factors such as introduced species (weeds and animals), disease (e.g. *Phytophthora cinnamomi*), recreation and climate change, etc.

The Upper Warren Fauna Monitoring protocols established as part of the WCRP provide a highly suitable model for consideration and development of more comprehensive and more informative fauna monitoring.

Target native species

Consider the collection of more detailed data from target species. This includes reproduction, health and condition and potentially reference samples for disease, particularly those that are relatively easy and cheap to collect and store (e.g. scats for endoparasites, ectoparasites, tissue for genetic research) as well as carefully considering the value of collecting other samples for monitoring, collaborative research and/or reference banking (e.g. blood for haemaparasites, haematology and/or biochemistry, and sera for parasites, viruses and other specific diseases.

Predators

As the situation with the woylie declines clearly demonstrates, it is not possible to associate the observed declines that occurred prior to the WCRP with possible changes in the abundance or activity of introduced predators such as the cat and fox. Furthermore, there is no direct means to verify that current predator control programs are effectively reducing targeted predators and not producing undesired responses such as the release of other problematic mesopredators. Without monitoring predators directly it is not possible to be responsive in a timely manner to any potential (foreseen or unforeseen) problems that may occur with predator control. Similarly without monitoring predators it is not possible to efficiently and effectively improve upon the current program. Monitoring of predators within an active adaptive management framework facilitates the capacity for ongoing improvement and the delivery of best-practice conservation and management as well as providing covariate data on a key known threatening process responsible for limiting and/or reducing native species such as the woylie and other threatened mammals.

Other covariates

Comparable covariate data to help explain changes in population of target species may include; diseases, resources, climate, competitors, prey, vegetation structure, health and floristics, etc.

Trigger points and reporting and response protocols

Irrespective of the type of monitoring conducted, in order to gain maximum value from the exercise it is critical to remain up-to-date with possible changes reflected in the data. This maximises the capacity to respond to new and emerging issues in a timely manner. There are several contemporary examples where monitoring data has detected substantial changes but have not elicited a timely and necessary response. In some cases this is a result of databases not being kept up-to-date with monitoring data collected in the field. In other cases the data has not been regularly interrogated. Worse still, in some cases substantial declines have been recognised but this information has not been investigated further or acted upon.

It is recommended that at the Departmental level, the revision or establishment of trigger points and associated reporting and response protocols be conducted. Trigger points (i.e. nominal quantifiable change thresholds) do not automatically necessitate an active response but should serve to alert the need to consider the available information more carefully before considering the need to respond further. In this context nominated trigger points do not need to be particularly precise and help to overcome many of the challenges associated with identifying appropriate thresholds including species specific considerations, and distinguishing ‘natural’ cycles and normal variation from something different. Data capabilities such as sensitivity measures of the data to detect change also need to be considered.

Obligatory annual reporting of fauna monitoring data would promote databases being kept up-to-date and can systematically address whether species have been observed declining. This could be associated with the annual reporting for Animal Ethics (whether or not the information was reviewed by the AEC or a separate body) given that it is a legitimate assessment of the justification of the fauna work being undertaken. Alternatively, it could be associated with the provision of Licences to take fauna, an expansion of the Science Division Annual Research Activity reporting across the Department or by some other means.

Concise protocols providing broad guiding principles and systems for managing cases when trigger points or problems are recognised would help to ensure that timely, efficient and appropriate responses to new information occur. These would outline who to report to, and who and how the new information should be considered in order to determine how best to respond.

8.3. Conservation management recommendations

8.3.1 Conservation status review

Completion of the review of the conservation status of the woylie considering all of the available data and information available to date is recommended. This review process should include State, Commonwealth and International programs where relevant. The development of effective recovery plans will be a natural and necessary progression if/when the change of conservation status decrees it.

8.3.2. Predator control

Improved efficiencies in the existing fox control program is highly recommended. This will help improve the efficacy of fox control with much of it incurring no or negligible additional costs to the current program. Reduced predation pressure on remnant woylies and other native fauna are clearly the key advantages of this. Operational improvements could include;

- reduce variability in the time intervals between baiting events from the current situation (1-9 months)
- strategic timing of baiting events to increase the efficacy of fox control, e.g. more closely associated with fox biology including when vixens support dependent young and seasonal peaks in dispersal
- clarify the effects of rainfall on bait efficacy and avoid baiting when conditions are likely to be problematic (e.g. heavy rainfall events forecast during or after baiting)
- routinely monitor predator activity/abundance in association with baiting activities within an active adaptive management framework to facilitate continued opportunities for improvement

Further detail is provided in the discussion of the PCS Predator section of this report (Section 4.4) and in this chapter above (‘Section 8.2.2). Some of these changes could be incorporated into the predator control experiment (AAM) discussed above.

8.3.3. Data and information management

Improvements to data and information management include;

- Consideration of the use of the ‘WCRP Operations Handbook’ as a basis for improvement to the protocols and procedures applicable to other programs.
- Keeping databases up-to-date and assessment of the data trends (facilitated by annual reporting obligations).
- Maintaining and developing the aggregated ‘Manjimup Fauna File’ (or equivalent) that functions as the central repository for all fauna data within a discrete geographic area. Progression toward a broader, perhaps single, Department-wide fauna database should be the ultimate aim.
- Improving data quality control through clear protocols and training, data-entry controls within the database, clear data crosschecking and validation procedures to identify and rectify errors.
- Improving data collection efficiency and quality by use of PDAs and software to record the raw data in the field.
- This is addressed in more detail in the ‘Research Recommendations’ section of this chapter (above) and the ‘improved fauna monitoring’ section (above)

8.4 Project management

Any continuation or development of the woylie conservation project will require a review of the project management. This is particularly important given the complexity of the project, the large number of people involved and the substantial ‘collaborative capital’ that has been established in phase 1. The appropriate project management framework will be dependent on the nature and extent of the support and priorities that are established for phase 2. Once this is clearer, careful consideration of the project management will be fundamentally important to the potential capacity of the project to function efficiently and effectively. Some general points for consideration include

- The WCRP Phase 1 project management framework more closely resembled a rapid (emergency) response model in which there was a high dependency on the generosity and goodwill of internal and external collaborators to contribute substantially (e.g. personnel, resources and intellectual property/expertise) in an informal manner. This has been the singularly most important factor in the success of the project to date. While flexibility remains important, fostering longer-term collaborations depends on ensuring and maintaining a satisfactory and fair balance on the currencies relevant to the collaborations and the mutualisms within in order to avoid over-exploitation.
- Project sustainability is fundamentally important to its success. Adequate personnel to meet the work loads and resources to manage the project in its own right are essential and in addition to those required to directly address the agreed research and management priorities.
- Adequate support and funding for phase 2 will be necessary as part of the woylie conservation response plan development.
- Excellent communication and data and information management between the many collaborating organisations and individuals is particularly important to the ongoing development of good collaborative relationships and project success.
- Media, public information and liaison, and engagement with interested community groups and individuals is particularly important to address adequately.
- Review processes (independent/external and internal) help ensure the project optimises its effectiveness and efficiency in a sustainable manner.

8.5. Species prognosis

Some brief notes on the consideration of the woylie declines within the context of the conservation outlook of the species and the capacity and relevance of the diagnosis efforts:

- The woylie has an excellent reproductive potential.
- The woylie has a proven record for a capacity to recover.
- The woylie is easily monitored (highly trappable).
- The woylie is not an intrinsically or evolutionary rare species (e.g. historic records report high densities).
- Manage the surviving remnant populations in a manner that facilitates their recovery (i.e. minimise manageable pressures (e.g. predation)), and the woylie's intrinsic capacities should be able to do the rest (i.e. species recovery should not in theory be as problematic as it is for other species).
- What keeps a population low may be different from what got them low in the first place – i.e. consider pragmatic indirect approaches – it may be more feasible/effective to manage declined populations rather than efforts to stop the declines.
- Understanding the causes of the declines is central to being able to make the best management decisions – this strategy is the most sound but is only practical if the research can provide reasonable answers within a reasonably short time frame.
- Understanding the cause(s) is necessary, whether or not the declines are 'natural' or due to factors that cannot be directly controlled. Irrespective of this, understanding the causes will have profound consequences on instructing how best to manage the species recovery and conservation in the short to long term. Even if the declines result from factors that may be considered impossible to address directly (e.g. inoculation for disease), there *will* be important and profound ways the risks and other related factors can be better managed that will i) improve the conservation of the species (e.g. quarantine and hygiene protocols (e.g. *Phytopthora*)), ii) determine the relative value of isolated and island populations, iii) the extent to which an emphasis is placed on the recovery of various populations, the establishment of new populations, etc.
- There is an increased vulnerability to population viability when numbers become extremely low (i.e. increased risk of local and species extinction).
- Rapid woylie population recovery is important to minimise genetic loss from bottlenecking etc, which will maximise the long-term conservation prospects
- Severe selective pressure has in all likelihood been imposed given the magnitude of the declines.
- Caution is needed when considering numbers of survivors without consideration of the genetic / longer-term conservation value (i.e. Dryandra, UW, Tutanning and translocated populations founded on large numbers / high genetic diversity) – i.e. there are some large populations with limited genetic/conservation value (e.g. some SA islands) that now constitute a substantial proportion of the survivors.
- The woylie declines provide an excellent model that will assist other species recoveries and decline diagnoses – probably the best marsupial available.
- National and International significance to decline diagnoses, species conservation and management, fauna monitoring, introduced predators and their control, wildlife disease and health surveillance, human health implications (zoonoses), and new and emerging diseases, etc.

8.6. References

Caughley, G. 1994. Directions in Conservation Biology. Journal of Animal Ecology **63**:215-244.

Lee, K. N. 1999. Appraising adaptive management. Conservation Ecology **3**:3.

Walters, C. J., and C. S. Holling. 1990. Large-scale management experiments and learning by doing. Ecology **71**:2060-2068.

Thylacinus cynocephalus, *'Head of a Male'* by John Gould, in *The Mammals of Australia* 1863

Lest We Forget